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CYTOLOGICAL CONDITIONS AND EVIDENCES FOR HYBRIDITY IN NORTH AMERICAN WILD ROSES[1]

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(WITH PLATES XVI–XIX AND FOUR FIGURES)

Introduction

Since the appearance of the outstanding papers of TÄCKHOLM (58, 59) on the cytology of *Rosa*, students of this perplexing genus have attempted to evolve satisfactory systems of classification of the multitudinous forms based on the varying chromosome numbers present (BLACKBURN AND HARRISON 4, HURST 31).

In previous studies on the cytology of our North American roses (TÄCKHOLM 59, PENLAND 46), the material has been obtained from the permanent collections in the various botanical gardens of Europe and the United States, the original stations for the material in the field being often unknown. It is not improbable that interspecific crosses take place between the roses growing together in a large wild rose collection, and since the botanical gardens customarily supply one another with seeds taken from their growing plants, it is possible that after one or two generations of such dispersal some of the "wild species" in a garden may be forms entirely unknown in the field. In the autumn of 1922, the Botanical Garden of the University of Michigan began to build up a collection of North

[1] Papers from the Department of Botany of the University of Michigan, no. 299.

American wild roses from known stations in this continent; this was done with the kind cooperation of many collectors, who responded generously to our appeals and whose efforts make these studies possible. I am particularly indebted to several members of the United States Forest Service, and to Mr. CLYDE LEAVITT of the Board of Railway Commissioners for Canada. Mr. C. C. DEAM and Mr. C. C. EPLING have been most generous in sending interesting and critical forms during the past five years. This collection is intended to aid in the systematic study of *Rosa*, the American forms of which are, many of them, just as polymorphic as are the European types whose chromosome complexes reveal their hybrid nature.

Other workers have reported races within some species of *Rosa* possessing different chromosome numbers based on multiples of seven. TÄCKHOLM, for example, found *Rosa acicularis* Lindl. from Russia to be octoploid, but his Swedish material comprised both hexaploid and octoploid races. PENLAND'S North European material was found to be hexaploid, as also Canadian material studied by HARRISON and BLACKBURN (**27**). TÄCKHOLM had some tetraploid specimens which ALMQUIST identified as *R. blanda* Ait.; PENLAND found *R. blanda* to be diploid. All the unquestionable forms of *R. cinnamomea* L. examined by TÄCKHOLM were diploid, whereas PENLAND (**46**) and HURST (**32**) place this species among the tetraploids. Although this is not an American species, it has escaped and has become established in several localities on this continent. The conclusion to be drawn from these findings is either that the chromosome number cannot be taken as a criterion for classifying *Rosa* forms, or else that the material has been incorrectly determined for the cytologists or by them.

In the present investigation many species have been examined cytologically from several parts of their natural ranges. A search was made for wild hybrid forms showing unpaired chromosomes, examples of which have not before been reported in American material. Artificial crosses have been made successfully between forms which differ in chromosome number. Unfortunately *Rosa* is a very unsatisfactory subject for genetical studies, both because of the difficulty in germinating the bony achenes, and because, except in a few Asiatic species, it takes three years for plants to reach maturity.

Material and methods

All the material for cytological study was taken from plants in the Botanical Garden of the University of Michigan, except material from a dozen early-flowering individuals which was kindly fixed by Dr. L. E. WEHMEYER in 1926 from plants in the Arnold Arboretum. The rose collection at Michigan was started at the end of 1922; few plants produced an abundance of flowers until 1926. Fixation was usually carried out in the afternoon, and meiosis was found to be just about as frequent on dull as on sunny days. Following the advice of Professor J. W. H. HARRISON and of Dr. C. C. HURST, the buds were fixed in Carnoy's solution. This fixing fluid has the advantage of rapid penetration, which is important in *Rosa* because the buds are fixed after merely slicing off the apex and base. Carnoy's solution causes a good deal of shrinkage, however, and in the summer of 1927 acetic-alcohol without chloroform was used, at the suggestion of Professor W. P. THOMPSON, who found that this gave a better result in his wheat material than Carnoy's solution. The acetic-alcohol swells the chromatin somewhat, facilitating observations. THOMPSON'S method (**60**) of examining the fixed material after it has stood in 85 per cent alcohol, staining in bulk with aceto-carmine, was tried. Although this method does not permit counts to be made, it enables buds in the reduction stage to be picked out before imbedding, thus saving unnecessary cutting.

Flemming's strong mixture, Zenker's solution, and Allen's modification of Bouin's fluid have also been tried. They all give less shrinkage than Carnoy's solution, but the penetration is poor. After these fixing fluids Haidenhain's iron-alum haematoxylin gives better results than after Carnoy's solution, when it sometimes fails to present good contrasts. The modification of Gram's stain as described by CLAUSEN (**9**) has been found very satisfactory, as has also plain gentian violet, particularly in the case of hybrid forms the protoplasts of which become much distorted by fixation.

For convenience prior to imbedding acetic-alcohol as a killing fluid is recommended, followed by staining with gentian violet; for detailed cytological studies in *Rosa*, strong Flemming or Allen's Bouin solution should be used, followed by iron-alum haematoxylin.

The fixed material was imbedded in paraffin. Sections of diploid

and triploid forms were cut 10–12 μ in thickness, those of tetraploids and higher polyploids at 12–14 μ, thus allowing some complete cells to appear on the sections and facilitating the counting of chromosome numbers.

Polyploid series

All North American wild roses heretofore examined cytologically fall into three groups: (1) the numerous diploid forms with fourteen somatic chromosomes and seven pairs at diakinesis; (2) the tetraploid group comprising *R. virginiana*, *R. carolina*,[2] *R. arkansana* and their allies, which have twenty-eight somatic chromosomes and fourteen pairs at diakinesis; (3) the *acicularis-nutkana* group which is hexaploid with forty-two somatic chromosomes and seven pairs at diakinesis. I have discovered an octoploid rose and also a few triploid and aneuploid forms on this continent.

Relative cell sizes in Rosa

Täckholm noticed that the size of microsporocytes at the stage of diakinesis is greater the larger the number of chromosomes, although he did not publish any measurements. The size of individual chromosomes appeared to him to be the same in all the forms of *Rosa*. Table I gives the diameter of microsporocytes measured at diakinesis. Four species or varieties from each group were selected from among diploid, tetraploid, and hexaploid species. These few measurements bring out the fact that diploid types have the smallest cells and hexaploids the largest, although there is considerable overlapping between the cell sizes at diakinesis in tetraploids and hexaploids.

Chromosome numbers

All the forms examined cytologically during the present study are included in the following list (table II). They are arranged according to their chromosome numbers, related types being grouped together in each class. The first column gives the species, accession number, and name of the original collector with date of collection.

[2] Cytologists should note the nomenclatorial confusion regarding *R. carolina* L. 1753, which is the correct name for *R. humilis* Marsh.; the name has unfortunately appeared in cytological literature as a synonym for *R. palustris* Marsh., the diploid species of the swamps of eastern North America (see Rydberg 49, and Fernald 22).

The second column gives the source of the material, usually the field collector's data. The third column contains the number of bivalent chromosomes at first reduction division; in the case of hybrid forms the number of unpaired, univalent, chromosomes present at diakinesis is placed after the number of bivalent chromosomes. The last column gives the number of chromosomes observed in somatic prophases. In the first column and immediately following the species

TABLE I

DIAMETER OF MICROSPOROCYTES AT DIAKINESIS

SPECIES AND ACCESSION NUMBER*	TYPE	KILLING SOLUTION	LARGEST DIAMETER OF CELLS IN μ
Rosa blanda 3505	Diploid	Carnoy's	6.4
R. pisocarpa 4634	"	Carnoy's	7.2
R. palustris 4412	"	Allen's Bouin	7.2
R. michiganensis 5891	"	Carnoy's	7.2
R. brachycarpa 5301	Tetraploid	Allen's Bouin	9.6
R. myriantha 3537	"	Carnoy's	9.6
R. obovata 2751	"	Allen's Bouin	8.0
R. carolina 5808	"	Carnoy's	8.0
R. engelmanni E5	Hexaploid	Carnoy's	8.8
R. acicularis var. lacorum 6007	"	Carnoy's	9.6
R. acicularis var. rotunda 3511	"	Strong Flemming	11.2
R. acicularis E3 (European)	"	Carnoy's	9.6

* Wherever "accession number" is referred to, the reference is to the serial accession numbers of the Botanical Garden of the University of Michigan. "Arn. Arb." numbers are those of the Arnold Arboretum. Nos. E 1, E 2, etc., are my cytological collection numbers for material fixed at the Arnold Arboretum.

name there are small letters which refer to the various kinds of meiotic irregularities observed. The key to these notations is placed at the head of the list.

Extrusion of chromatin has been observed in many cytological preparations of the sporocytes of plant genera. Chromatin or chromosomes are sometimes extruded into the cytoplasm, as found by TISCHLER (61), YASUI (67), LONGLEY (41), HURST (31); or chromatin may pass through the wall into an adjacent sporocyte, as shown by DIGBY (16) in *Galtonia candicans*, by GATES (24) in *Oenothera gigas*, and by WEST and LECHMERE (64) in *Lilium candidum*. This has been observed frequently in my material, especially in the prophase stages of meiosis (figs. 6, 15, 51), and is indicated by letter *b* in the first column. The significance of the phenomenon is

not understood, but it is regarded by several cytologists (TISCHLER **62**, SAKAMURA **50**) as being an indication of degeneration. Recently HURST (**31**) has used the occurrence of chromosome extrusion in *Rosa* as an argument in support of his theory of the origin of rose types with lower chromosome numbers from the higher polyploids.

In some roses it has been observed that occasionally a microsporocyte will give rise to only two spores (diad) instead of the normal four (tetrad) after meiosis. This has been indicated by *h* in table II.[2a]

Cytological results

DIPLOID SPECIES

Rosa bracteata Wendl.—This rose, belonging to the small Asiatic section Bracteatae, was not obtained by TÄCKHOLM for his extensive studies. It has become thoroughly naturalized in southeastern United States, where it is common. A plant collected in northwestern Mississippi and grown in the greenhouse proved to be diploid, with seven pairs at diakinesis (fig. 1).

Rosa setigera Michx.—The single American representative of the section Synstylae is very constant in its morphological characteristics, varying chiefly in the amount of pubescence on the foliage. A specimen of the pubescent var. *tomentosa* T. & G. (*R. rubifolia* R. Br.) from Ohio confirmed TÄCKHOLM'S observation, showing seven pairs at diakinesis.

Rosa gymnocarpa Nutt.—This species, reported by TÄCKHOLM to have fourteen somatic chromosomes, was found to have seven pairs at the first meiotic division (fig. 2). Material has not yet been obtained from any other member of the section Gymnocarpae.

Rosa palustris Marsh.—The tall swamp rose of eastern and southeastern North America is diploid, as has already been shown by others. It is the largest rose in Michigan, excepting *R. setigera*, and

[2a] LIST OF CHROMOSOME NUMBERS.—*Explanation of notations:* (a) affinity between members of some pairs at first meiotic reduction weak, resulting in some univalent chromosomes at first anaphase (fig. 20); (b) much extrusion present in material; (c) two ovules observed in one ovary; (d) lagging of some chromosomes at meiotic anaphases (figs. 54, 59); (e) polyspory observed at tetrad stage of microsporogenesis (fig. 48); (f) meiosis not synchronous in sporocytes of one microsporangium; (g) rings of four chromosomes observed (figs. 39, 46); (h) diad groups found among tetrads of pollen (fig. 32).

TABLE II

Species, accession no., and original collector	Origin	Haploid no.	Somatic no.
	Diploid types		
R. bracteata Wendl. 3676, L. E. Wehmeyer, 1923	Miller, De Soto Co., Miss.	7	.....
R. setigera Michx. 3585, unknown.	Ohio	7	.....
R. gymnocarpa Nutt. 4006, C. C. Epling, 1923	Priest River, Idaho	7	.....
Cinnamomeae			
R. cinnamomea L. (b) 6082, Moses Gomberg, 1924	Keene Valley, Essex Co., N.Y.	7	.....
R. pisocarpa A. Gray 4634, R. M. Reid, 1923	Castella, Shasta Co., Calif.	7	.....
R. salicetorum Rydb. 3530, Ehlers and Erlanson, 1923	Copeland, Boundary Co., Idaho	7	.....
R. puberulenta Rydb. 5594, E. G. Wiesehuegel, 1924	Wyo. Natl. For., Sublette Co., Wyo.	7	.....
R. macounii Greene 3820, S. Dowding, 1923	Edmonton, Alberta	7	14
R. woodsii Lindl. 3298 (plant D), F. B. Cotner, 1923	Bozeman, Gallatin Co., Mont.	7	14
R. woodsii Lindl. (a) (e) 4477 (plant N4) Edine Binney, 1923.	Fort Pierre, Stanley Co., S.D.	7	14
R. woodsii Lindl. (b) 3255, A. O. Garrett, 1923	Salt Lake Co., Utah	7	.....
R. woodsii Lindl. 8152, C. O. Erlanson, 1925	So. of Canyon City, Fremont Co., Colo.	7	14
R. pyrifera Rydb. (e) (f) 6610 (2 individuals), A. O. Garrett, 1925	Salt Lake Co., Utah	7, 8	14, 16
R. ultramontana Wats. 5293, W. H. Ransome, 1924	Spokane Co., Wash.	7	.....
R. fendleri Crépin (a) 3298 (plant A), F. B. Cotner, 1923	Bozeman, Gallatin Co., Mont.	7	.....
R. fendleri Crépin 5679, E. L. Perry	Santa Fé Natl. For., Sandoval Co., N.M.	7	.....
R. hypoleuca W. & S. 8154, C. O. Erlanson, 1925	Arkansas River, Fremont Co., Colo. (alt. 5500 ft.)	7	14
R. granulifera Rydb. (b) 5925, W. A. Archer, 1924	Black Creek, Sierra Co., N.M.	7	.....
R. subblanda Rydb. 3608, L. E. Wehmeyer, 1923	Roanoke, Huntington Co., Ind.	7	.....
R. blanda Ait. (d) (e) 4071, H. H. Bartlett, 1923	St. Ignace, Mackinac Co., Mich.	7	.....
R. blanda Ait. 3505, E. W. Erlanson, 1923	Bliss Woods, Kane Co., Ill.	7	.....
R. blanda Ait. 3942, H. H. Bartlett, 1923	Lakeland, Livingston Co., Mich.	7	.....
R. blanda var. hispida (d) 5771 (plant B), C. G. Harrold, 1924.	Gimli, Lake Winnipeg, Manitoba	7	.....

TABLE II—*Continued*

SPECIES, ACCESSION NO., AND ORIGINAL COLLECTOR	ORIGIN	HAPLOID NO.	SOMATIC NO.
R. blanda var. hispida (a) (b) (d) 5773, C. G. Harrold, 1924	Gimli, Lake Winnipeg, Manitoba	7	
R. blanda var. hispida (d) 2681, E. S. Reynolds, 1922	Agric. College, Cass Co., N.D.	7	
R. blanda var. hispida 2693, Helen Stegenger, 1922	University, Grand Forks Co., N.D.	7	
R. blanda var. hispida 4167, A. H. W. Povah, 1923	Beach, Cook Co., Ill.	7	
R. blanda var. hermanni 2686, F. J. Hermann, 1922	Laurium, Houghton Co., Mich.		14
R. blanda glandulosa (a) (b) (d) (e) 3753, J. H. Ehlers, 1923	Prentis Bay, Mackinac Co., Mich.		
Parent plant B		7	14
Seedling plant 2/N38		7	
Seedling plant 5/N3		7	
Seedling plant 7/N25		7	
Seedling plant 7/N36		7	
Seedling plant 7/N37		8	16
Seedling plant 7/N57			14
Seedling plant 7/N62			14
Seedling plant 9/N1		7, 8	15
Seedling plant 9/N9		7	
R. michiganensis (b) (c) (d) (e) 5890, E. W. Erlanson, 1924	Douglas Lake, Cheboygan Co., Mich.	7	
R. acicularioides (a) (d) (e) 8261, H. H. Bartlett, 1925	Ogdensburg, St. Lawrence Co., N.Y.	7	14
R. schuetteana (d) (e) 5891, C. and E. Erlanson, 1924	Douglas Lake, Cheboygan Co., Mich.	7	
Carolinae			
R. palustria Marsh. 4412, E. E. Gunther, 1923	Spider Lake, Grand Travers Co., Mich.	7	
R. palustris var. inermis (b) (d) (e) 5714, H. H. Bartlett, 1924	Quanicassee, Saginaw Bay, Mich.	7	
R. foliolosa alba (d) (e) 9525, Ralph Shreve, 1926	Wetumka, Hughes Co., Okla.	7	
R. foliolosa alba (d) 9526, Ralph Shreve, 1926	Montagu Co., Texas	7	
R. subserrulata (a) (d) 9528, Ralph Shreve, 1926	W. Fork, White River, Okla.	7	
R. rugosa (hyb.)×?blanda (×R. tetonkaha) (b) (d) 5328, N. E. Hansen, 1925	Hort., Brookings, S.D.	7	
	TRIPLOID TYPES		
(?) R. virginiana×palustris (a) (b) (d) (e) (f) (g) 2872, E. C. Robbins, 1922	Highlands, Macon Co., N.C.	7u7₁	21
(?) R. blanda×carolina (a) (b) (d) (e) (f) (g) 2949, W. E. Manning.	Esty's Glen, Ithaca, N.Y.	7u7₁	21

TABLE II—*Continued*

Species, accession no., and original collector	Origin	Haploid no.	Somatic no.
	Tetraploid types		
Pacific Coast Cinnamomeae			
R. californica (d) (e) (g) 5849, W. A. Archer, 1924	Toulomne River, Modesto, Calif.	14	.....
R. aldersonii (b) (d) (g) 5367, C. C. Epling, 1925	Griffith Park, Los Angeles, Calif.	14	.....
R. brachycarpa (a) (d) (g) 5301, H. Baer, 1924	Temescal Canyon, Elsinore, Calif.	14	.....
R. myriantha (a) (d) (g) 3537, Ellen Bach, 1923	Nevada City, Nevada Co., Calif.	14	28
Carolinae			
R. virginiana (d) (g) 2790, M. Bilon, 1922	Madison, New Haven Co., Conn.	14	.....
R. virginiana (d) (e) 6071, P. F. Weatherill, 1924	Orr's Island, Cumberland Co., Me.	14	.....
R. virginiana (b) (d) (g) 3236 (plant C) Hort.	Naperville Nurs., Naperville, Ill.	14	.....
R. obovata (e) (g) (h) 2651, E. W. Erlanson, 1922	Ann Arbor, Washtenaw Co., Mich.	14	.....
R. deamii (d) (e) (g) 3779, C. C. Deam, 1923	Goldsmith, Tipton Co., Ind.	14	.....
R. lyoni (e) 2883, C. C. Deam, 1923	St. For. Res., Clark Co., Ind.	14	.....
R. lyoni (e) 3168, L. F. Brumfield, 1923	A. & M. College, Oktibbeha Co., Miss.	14	.....
R. lyoni (b) (e) (g) 3203, J. T. Buchholz, 1923	Fayetteville, Washington Co., Ark.	14	.....
R. carolina L. (d) (g) 5808, H. H. Bartlett, 1924	Camp Custer, Kalamazoo Co., Mich.	14	.....
R. carolina L. (g) 5291, David Boot, 1924	Iowa City, Johnson Co., Iowa	14	.....
R. carolina var. litoralis (e) 2654, A. H. W. Povah, 1922	Evanston, Cook Co., Ill.	14	.....
R. serrulata Raf. (d) (e) (g) 5566 (plant A), F. K. Sparrow, 1923	Montgomery Co., Md.	14	.....
R. serrulata Raf. (e) 3938, Bartlett & F. C. Blanchard, 1923	Lakeland, Livingston Co., Mich.	.....	28
R. subserrulata (a) (d) (e) 6072, W. A. Archer, 1924	Rogers, Benton Co., Ark.	14	.....
R. petiolata (d) (e) 3669, L. E. Wehmeyer, 1923	Boss, Dent Co., Mo.	14	.....
R. rudiuscula (a) (d) (e) (f) (g) 3501, E. W. Erlanson, 1923	St. Charles, Kane Co., Ill.	14	.....
R. rudiuscula (a) (e) (g) 4001, C. C. Deam, 1923	Crown Point, Lake Co., Ind.	14	28
R. rudiuscula (e) 4293, B. F. Bush, 1923	Greenwood, Jackson Co., Mo.	14	.....
Cinnamomeae			
R. bushii Rydb. (d) (e) (g) 3080, B. F. Bush, 1923	Courtney, Jackson Co., Mo.	14	.....
R. alcea (h) 3571, S. Dowding, 1923	Calgary, Alberta	14	.....

TABLE II—*Continued*

Species, accession no., and original collector	Origin	Haploid no.	Somatic no.
R. subglauca (a) (d) (e) 3492, A. H. Brinkman, 1923	Craigmyle, Alberta	14	28
R. suffulta (a) (b) (d) (e) (g) 2682, E. S. Reynolds, 1922	Agric. Coll., Cass. Co., N.D.	14	.....
R. suffulta (d) (e) (g) 2692, H. Stegenger, 1922	University, Grand Forks Co., N.D.	14	.....
R. suffulta (d) (e) 2694, H. Stegenger, 1922	University, Grand Forks Co., N.D.	14	.....
R. suffulta (a) (e) (g) 5598, U.S. For. Service, 1924	Monument, El Paso Co., Colo.	14	.....
R. suffulta (a) (d) (e) (g) 5615, W. A. Archer, 1924	Larkspur, Douglas Co., Colo.	14	.....
R. suffulta (g) 5705, E. V. Anderson, 1924	Concord, Dixon Co., Neb.	14	.....
R. suffulta (d) 2897b, H. S. Conard, 1922	Grinnell, Poweshiek Co., Iowa	14	.....
R. suffulta (d) (e) (g) 3001, F. C. Gates, 1923	Riley Co., Kans.	14	.....
R. suffulta var. valida (a) (d) (e) (g) 4459, A. and P. Hamilton, 1923	Rockport, Atchison Co., Mo.	14	.....
R. arkansana 5771 (plant G) C. G. Harrold, 1924	Winnipeg, Manitoba	14	.....
R. ratonensis (b) (e) (g) 8114, C. O. Erlanson, 1925	Colfax Co., Raton Pass, N.M. (alt. 8800 ft.)	14	.....
R. relicta (g) 8320, C. & E. Erlanson, 1925	Bliss Woods, Kane Co., Ill.	14	.....
	Hexaploid types		
R. nutkana Presl. 4004, C. C. Epling, 1923	Priest River, Idaho	21	.....
R. macdougali (d) (e) E. 8, Arn. Arb. 12352	Idaho	21	.....
R. spaldingii 5549, A. A. Griffin, 1924	Naches River, Yakima Co., Wash.	21	.....
R. spaldingii 5298, A. A. Griffin, 1924	Tieton River, Yakima Co., Wash.	21	.....
R. spaldingii E. 4, Arn. Arb. 11116	Pullman, Whitman Co., Wash.	21	.....
R. spaldingii E. 7, Arn. Arb. 1268.	Idaho	21	.....
R. spaldingii E. 10, Arn. Arb.	Elk Mt., Idaho Co., Idaho	21	.....
R. engelmanni 3254 (plant D), C. C. Moore, 1923	Missoula, Missoula Co., Mont.	21	.....
R. engelmanni, B. B. Kanouse, 1923			
3719 (e)	Medicine Bow Mts., Albany Co., Wyo.	21	.....
3721/A		21	.....
3722 (e) (b)		21	.....
3723		21	.....

TABLE II—*Continued*

Species, accession no., and original collector	Origin	Haploid no.	Somatic no.
R. engelmanni (e) E. 5, Arn. Arb. 9052 A. Rehder, 1916	Pikes Peak, El Paso Co., Colo. (alt. 9000 ft.)	21	.....
R. butleri Rydb. (a) (e) 8271, C. O. Erlanson, 1925	Sangre de Cristo Mts., Custer Co., Colo. (alt. 8400 ft.)	21	.....
R. underwoodii Rydb. 8270, C. O. Erlanson, 1925	San Isabel Nat. For., Custer Co., Colo. (alt. 8400 ft.)	21	.....
R. acicularis var. lacorum 6008, E. W. Erlanson, 1924	Prentis Bay, Mackinac Co., Mich.	21	.....
R. acicularis var. rotunda 3511, C. O. Erlanson, 1923	Douglas Lake, Cheboygan Co., Mich.	21	.....
R. acicularis var. bourgeauiana (b) (e) (g) 2502, H. H. Bartlett	Mackinaw City, Cheboygan Co., Mich.	21	.....
R. acicularis var. sayiana, J. H. Ehlers, 1922 (a) (b) (d) (e) (f) 3754 (plant A), 3754 (plant D)	Prentis Bay, Mackinac Co., Mich.	21	.....
R. acicularis var. sayiana 6007, C. & E. Erlanson, 1924	Scotty Bay, Mackinac Co., Mich.	21	.....
R. acicularis Lindl. E. 3 Arn. Arb., 789-5	Hort, Kew	21	.....
R. acicularis var. nipponensis, (d) E. 14, Arn. Arb., 7839	Hort. Chénault, France	7	.....
	Octoploid type		
R. acicularis var. lacorum 6447, C. H. Morgan, 1924	Fairbanks, Alaska	28	.....

sometimes reaches over 7 feet in height. Morphologically it is the most highly evolved of the Carolinae-Cinnamomeae east of the Rocky Mountains. In northern Michigan and Wisconsin it has early-flowering races. These are also diploid, and were first recognized by Schuette. Some of these seem to be due to hybridization between *R. blanda* Ait. and *R. palustris*, and are taxonomically distinct. Three of them have already been described as *R. michiganensis*, *R. schuetteana*,[3] and *R. blanda* var. *hermanni* (Erlanson

[3] *Rosa schuetteana* resembles *R. carolina* L. in some respects, and was at first reported as that species (18). Cytologically it exhibits few irregularities, and has less than 10 per cent of sterile pollen. Since the tetraploid *R. carolina* has not been found in Cheboygan County, Michigan, it seems more probable that this species is descended from *R. palustris* × *R. blanda*, both of which are diploid forms, instead of from *R. carolina* × *palustris*. The flowering time of *R. schuetteana* is intermediate between those of *R. blanda* and *R. palustris*. This is one case in which cytological examination has aided in the classification of a rose form.

19). These plants exhibit a few irregularities in meiosis, chiefly due to lagging chromosomes during the first and second anaphases. There is a certain amount of polyspory, but the proportion of dwarf pollen grains is small, no larger than in the two hypothetical parents.

HURST (31) thinks that there are five primary groups of diploid species in *Rosa*, which he designates as AA, BB, CC, DD, and EE, each with a distinct set of linked factors. In his report before the Genetical Society at Cambridge, England (32), he designated *R. palustris* as tetraploid AADD and *R. blanda* as diploid DD. *R. palustris*, however, is undoubtedly a diploid species and is typically very distinct from *R. blanda*. These two species would be expected to belong to different primary groups.

DIPLOID SPECIES OF CINNAMOMEAE

A semidouble form of *Rosa cinnamomea* found growing wild in New York proved to be diploid. This record is of interest owing to the conflicting chromosome counts which have been obtained for this species. HURST (32) reported that there exist tetraploid varieties of this species which are "quadrivalent in synapsis."

TÄCKHOLM'S findings as to the diploid nature of *R. pisocarpa* A. Gray[4] (fig. 3), *R. woodsii* Lindl. (fig. 4), and the closely related *R. fendleri* Crép. and *R. macounii* Greene have been confirmed in wild material. Other species from the Rocky Mountain region, related to these, which have been found to be diploid, are *R. ultramontana* S. Wats., *R. salicetorum* Rydb.[5] (fig. 5), *R. pyrifera* Rydb., and *R. puberulenta* Rydb. These are all tall roses and are characteristic of the waterways. So far as I know, the largest wild roses in any region in this country are the diploid forms, the polyploid forms being as a rule low shrubs growing to 4 feet in height, but usually under 3 feet high in nature.

Rosa granulifera Rybd. and *R. hypoleuca* Woot. & Stand., both

[4] Specimens of *R. pisocarpa* in most herbaria are of two distinct types: (a) true *R. pisocarpa* with straight ascending infrastipular prickles, oval leaflets, and small hips in crowded corymbs; (b) plants with more scattered weak armature or none, more narrowly oval or obovate leaflets with triangular coarser teeth, larger flowers and hips. These latter belong to the *R. macounii* complex.

[5] This specific name was misspelled in the original publication as *R. salictorum* (RYDBERG 48).

closely related to *R. fendleri*, are diploid as would be expected (figs. 6, 7).

In the group of *R. woodsii*, reduction takes place in the pollen mother cells when the buds are very small, about 2 mm. in diameter by 5 or 6 mm. long. In the following diploid forms the buds are large, about 4.5 mm. in diameter and 8 mm. or so long, when pollen formation takes place.

As already determined by PENLAND (**46**), *R. blanda* is a regularly diploid species. I have not been able to find any form of this species showing twenty-eight somatic chromosomes, and do not think that TÄCKHOLM'S tetraploid specimens belonged to this species. They may have been some of the forms near *R. bushii* Rydb. and *R. rudiuscula* Greene. ALMQUIST determined them as *R. acicularis*.

Rosa subblanda Rydb., a glabrous relative of *R. blanda*, is diploid, as are all the forms of *R. blanda* which have been examined (fig. 8). There are numerous bristly types of *R. blanda*, corresponding to *R. blanda* var. *hispida* Farwell, which are found in widely separated parts of the range of the species, and nearly all exhibit some irregularities during meiosis. The most frequent irregularities are the failure of one or two pairs of chromosomes to be included in the first spindle (fig. 9), or the lagging of some pairs at first anaphase. Rarely at the second division a small extra spindle is observed. Some chromosomes may lag at this division also, and become inclosed in subsidiary nuclei giving polyspory. It is not usual to find more than one extra grain from a pollen mother cell, and counts of mature pollen reveal an appreciable and variable proportion of shriveled grains, from 6 to 33 per cent. These bristly varieties of *R. blanda* when collected in the western parts of its range are usually named *R. macounii*. *R. macounii* is probably a composite species, consisting in part of pubescent varieties of *R. woodsii* (most botanists so interpret *R. macounii*), and in part of more robust forms which should be placed in *R. blanda* var. *hispida*.

R. acicularioides belongs in the group of *R. blanda*, and there are cytological indications that it is of hybrid origin. A specimen from New York was found to be diploid, but some of the chromosomes show a weak affinity at meiosis and in some cells may fail to pair at all. Fig. 20 shows a first meta-anaphase in which four of the chromo-

somes have apparently failed to pair, the members of one pair lying at widely different depths on the spindle. The daughter nuclei will each receive seven chromosomes, however, and several sister interkinesis nuclei have been found, each one of which contains seven chromosomes and a nucleolus. Somatic cells show fourteen chromosomes (fig. 21). The plant from which the fixations were made produces abundant fruit, although castrated buds do not develop. If, under HURST'S scheme, it contains two similar differential septets of chromosomes, let us say DD, one would expect to get complete pairing between the seven homologous pairs; if on the contrary it contains two different septets, say C and D, it should be sterile under this scheme.

DIPLOID HYBRIDS

R. blanda glandulosa Schuette

A culture of over 200 seedlings grown from seed of four separate plants of *R. blanda* var. *glandulosa* Schuette is growing at the University of Michigan Botanical Garden. The parent plants were collected in Mackinac County, Michigan, by Dr. J. H. EHLERS in 1923 (accession no. 3753), and the seeds of each were planted separately when they were received from the field. The seeds germinated in 6 months and throughout the first season the seedlings were fairly uniform. These plants came to maturity in 1926 and by then showed great variety. The stems now vary from almost unarmed to densely bristly throughout; in color they vary from red-brown to greenish brown. The hips vary from long-pyriform to globose, and may be either erect or pendulous when ripe. Several of the plants have glandular-compound teeth on the leaflets, as in *R. acicularioides* Schuette. Several plants did not flower in 1926, their third year of growth, and a few of these still failed to produce flowers in 1927. About half a dozen plants were badly cut back by the winter of 1925–1926, in spite of the fact that the parent plants came from latitude 46° north. The majority of the seedlings flowered profusely in 1926 and 1927, at the same time as *R. blanda*, and produced a full crop of large hips full of good achenes. *R. blanda* var. *glandulosa* is characterized by having pyriform hips, and has been called *R. acicularis* × *blanda* by RYDBERG (**48**). The morphology of the culture

just described would seem to confirm this diagnosis. Table III analyzes the progeny of the four plants for some characteristics often used to distinguish species in *Rosa*. The majority of the plants bear ellipsoid hips, although these may vary from subglobose to ellipsoid on the same plant. As a rule those individuals which have gland-compound teeth do not have them exclusively; those exhibiting any have been placed in the category "gland-compound." During the

TABLE III

VARIATION IN PROGENY OF FIELD-POLLINATED ROSES

Numbers of specimens exhibiting certain characteristics in cultures grown from seeds of four individual plants of *Rosa blanda* var. *glandulosa* Schuette (no. 3753), from Mackinac County, Michigan; seeds taken from parent plants when received from the field in 1923

CULTURE AND TOTAL NUMBER OF PLANTS	CHARACTERISTICS								
				Leaflet teeth		Hip shape		Sepals	
	Semi-hardy	No flowers in 1926 only	No flowers in 1926 or 1927	Simple	Gland-compound	Sub-globose	Ellip-soid	Glan-dular	Smooth
3753/2 (50 offspring)	3	11	4	41	9	11	32	29	14
3753/5 (34 offspring)	0	10	3	32	2	.....	.....	.....	.....
3753/7* (Total progeny 133, data taken on 75) . .	14 in 133	15	16	67	8	32	12	32	13
3753/9 (16 offspring)	0	1	1	7	9	1	10	8	1

*The progeny of 3753 plant 7 contained five plants that bore few flowers and no fruit in 1927, two plants that bore only one deformed hip in 1927; plant N54 had glabrate leaflets and several of the hypanthia aborted after anthesis.

first season the seedlings all had glabrous leaflets with glandular-compound teeth, two characteristics which seem to be universal among *Rosa* seedlings, although in some species the leaflets may become eglandular after the first two or three leaves are produced. In the seedling plants of no. 3753, some show glandular-compound teeth only on the leaves at the tips of turions and vigorous lateral shoots, toward the end of the season. In habit and flowering time most of these plants are like *R. blanda*. They also possess the corymbose inflorescence of that species. A few plants show partial sterility, in that only a few hips are produced and these contain only three or four achenes. One plant (9/N5) flowered but produced no fruit in 1927. Only one plant in the whole culture (7/N117) flowered

appreciably before the rest and showed habit and foliage typical of *R. acicularis*.

Thus far one of the parent plants and nine of the seedlings have been examined cytologically and all but two have been found to be diploids with fourteen somatic chromosomes (fig. 10). Pollen counts made on two of the apparently fertile plants showed 17.6 and 25 per cent of dwarf shriveled grains. The fact that the fixed material of culture 3753 exhibits a great deal of shrinkage, distortion, and chromatin extrusion strengthens my belief that it is of hybrid origin. (CLAUSEN [9] found that the distortion caused by fixation was particularly troublesome in his hybrid *Violae*.) At diakinesis there is a marked delay in pairing among some of the chromosomes (figs. 11, 12), some of which may still be unpaired at the time of heterotypic metaphase. The metaphase plates of the second meiotic division have been found showing seven chromosomes in both daughter nuclei. Some polyspory is evident, as would be expected from the condition of the pollen.

Two of the semihardy plants, which produce no flowers and have only a weak vegetative growth, were brought into the greenhouse, and root tips were fixed in Flemming's fluid, half strong. These showed fourteen somatic chromosomes (fig. 13).

Individual with fifteen chromosomes

Only two of the plants so far examined (culture 3753, plants 9/N1 and 7/N37) showed any extra chromosomes. One of these (plant 9/N1), a plant with glandular-compound teeth, has fifteen somatic chromosomes (fig. 14). At diakinesis there may be more than one univalent chromosome; fig. 15 shows three. There is some lagging at both meiotic divisions. The seven bivalent chromosomes and one univalent may lie on the first spindle (fig. 16), or some may not be included in it. Fig. 17 shows three pairs off in the cytoplasm. Interkinesis nuclei show both seven and eight chromosomes; in fifty cells thirty-five contained seven and fifteen eight. Fig. 18 shows two daughter nuclei at second metaphase. The extra chromosome has already divided and may have done so at first anaphase. In fig. 19 both the daughter nuclei show seven chromosomes at second metaphase, and the extra chromosome has apparently been extruded

and lies on the surface of the shrunken protoplast. This plant then probably produces gametes, mostly with seven, but occasionally with eight, chromosomes. It is a vigorous bush over 4 feet high and bore large crops of tapering hips in 1926 and in 1927.

R. blanda×*acicularis*

The plants of culture 3753 apparently are a later generation, perhaps many times removed, from a cross between the diploid *R. blanda* and the hexaploid *R. acicularis* of northern Michigan. Such a cross would be expected to give seven bivalents and fourteen univalent chromosomes at reduction division of the first hybrid generation. These hybrids would be expected to be highly sterile, only those gametes receiving few or no univalent chromosomes being able to function. It is possible that the elimination of the univalents through lagging on the spindles at meiosis led to the establishment of the largely stable and fertile diploid type resembling the diploid parent. Such reestablishment of constant types in later generations from crosses between individuals with different chromosome numbers has been found to occur in *Triticum* (KIHARA **38**), in *Oenothera* (BOEDIJN **6**), in *Papaver* (by a back cross to one of the parent types) (YASUI **67**), and has been suggested by HEILBORN (**29**) as the way in which *Draba magellanica* with twenty-four chromosomes originated.

The great variation in the culture and the presence of some plants with glandular leaflets and pendulous pyriform hips (characters absent in *R. blanda*) suggest affinities with *R. acicularis*, and seem to show that in the original cross seven chromosomes from each parent paired, as would be required by HURST'S scheme; but according to that scheme the progeny of such a cross would be sterile. There are in this culture, from four wild unguarded plants, forms that would be named *R. blanda*, *R. blanda* var. *glandulosa*, *R. blanda* var. *hispida*, and *R. acicularioides*, as well as sterile forms.

During the summers of 1926 and 1927 the cross *R. blanda*×*R. acicularis* and its reciprocal were successfully produced several times. The former cross yields as good a crop of achenes as any cross I have made. *R. woodsii* crossed with hexaploid types has also given good fruit. It may be significant that crosses between diploid and poly-

ploid roses are more often successful when the diploid is the female parent. Attempts to germinate these seeds have so far yielded only one seedling, but there is every indication that these crosses occur and are successful in nature.

It seems highly probable that some of the North American diploid roses have arisen in the manner suggested from crosses between some other diploid with a polyploid form. Such an origin would complicate matters, and would be more in accord with the taxonomic confusion proverbial in this genus than an origin of diploids from polyploids, according to HURST's theory, by the simple loss of sets of seven chromosomes. The variability of the diploid descendants of such crosses is likely to be great, and they can be expected to cross with each other and with the parent forms in the field, to continue to segregate and to give fresh combinations.

The first generation offspring of crosses between parents with different chromosome numbers are probably few and highly sterile, hence inconspicuous in the field. The flowering times of our hexaploid, diploid, and tetraploid roses are characteristic and often distinct, and wide crosses are presumably infrequent in nature. However, the possibility of the production of strong fertile descendants from such crosses in two or three generations permits the taxonomist to ascribe to hybridization the many intermediate forms so notorious in *Rosa* throughout the world.

R. acicularis var. nipponensis Koehne.

It is pertinent to record here that two plants from two different cultures of *R. acicularis* var. *nipponensis* growing at the Arnold Arboretum have been examined cytologically. One is diploid with seven pairs at diakinesis, producing good fruit; the other is almost sterile, seldom producing fruit and the fixed material is very badly shrunken, having seven pairs and also some univalent chromosomes at diakinesis. The plants were grown from seed sent by CHÉNAULT from France. Material that TÄCKHOLM obtained from Kew as this variety he found to be tetraploid. The seeds of *R. acicularis* var. *nipponensis* were distributed to the botanical gardens of Europe by the Hortus Petropolitanus about 1870 (WILLMOTT 65). It may be that since then the plants have crossed with others having different

chromosome numbers, until finally a fertile diploid form has been obtained in the same manner that the stable diploid seems to have been produced from *R. acicularis*×*blanda* in Michigan.

R. rugosa hyb.×*blanda* (?)

The Tetonkaha rose originated by HANSEN (**26**), supposedly from the foregoing cross, is diploid, as would be expected from a cross between two diploid species. HURST (**32**) has reported this cross, and according to his scheme it is CC×DD and should be sterile, unless it became fertile by a somatic duplication, thereby becoming tetraploid CCDD. The Tetonkaha rose produces large hips resembling those of *R. rugosa*, and apparently crosses with other roses, since seed obtained from another plant growing at Washington, D.C. gave large seedlings with the habit of *R. multiflora* or *R. arvensis*. These latter, however, do not set fruit. Apparently crosses between very distinct diploid species may give fertile offspring.

MEIOTIC IRREGULARITIES IN DIPLOIDS

The early history of meiosis in all the species examined has corresponded with that described by BLACKBURN and HARRISON (**4**) for *R. arvensis*. The chromosomes are arranged end to end, and sometimes pairing is considerably delayed after strepsinema during condensation, so that strings of chromosomes appear (fig. 5). Frequently the members of some pairs become attached by their ends alone, thus forming a ring (fig. 12), these configurations recalling similar ones in *Oenothera*. Frequently two chromosomes in a nucleus will delay pairing until the first spindle is formed. In some cases, although all the chromosomes may come to lie on the equator of the first spindle, the members of one pair may fail to associate with each other (fig. 20). It is noticeable that condensation proceeds irregularly among the chromosomes after fragmentation of the spireme, so that it is not unusual to find the majority of the pairs well condensed, with one or two still long and threadlike. This state of affairs is also true among the tetraploids (fig. 38 *A*).

Meiosis in North American diploid roses is seldom completely regular as it was found to be in *R. arvensis* by BLACKBURN and HARRISON. Most of the forms studied resemble cytologically the

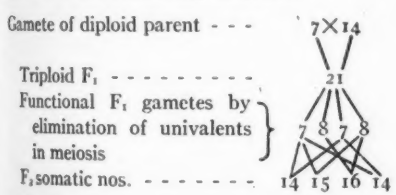
diploid hybrids examined by TÄCKHOLM. Sometimes one or more pairs of chromosomes fail to reach the first spindle (figs. 9, 17), and may lag behind the others at first anaphase. The interkinesis nuclei usually receive seven chromosomes, but there may be more lagging at second anaphase. These irregularities are reflected in the presence of polyspory (fig. 24) and in the pollen counts (table IV), which show a considerable proportion of shriveled grains for species in which PENLAND considers hybridization scarcely to exist.

Extrusion of chromosomes, upon which HURST lays so much stress, is frequent in all stages of meiosis, and is more noticeable in the more sterile plants than in others. In how far this phenomenon is an artifact is not yet known; my material seems to show that in many cases it is probably due to imperfect fixations. Fig. 6 shows two contiguous cells in which extrusion at diakinesis is commencing; the nuclear membrane in both cells has been drawn out nearer to the edge of the protoplast and has been broken through in the same relative position, which suggests a too sudden penetration of the fixing fluid from one side. The number of chromosomes extruded may vary, as found by LONGLEY (41) in triploid *Rubus* species. Occasionally all the chromosomes of many cells in a loculus are found to be extruded, or more rarely all the chromosomes of a cell are extruded into the cytoplasm of a neighboring pollen mother cell (fig. 15). Even according to HURST's theory there does not seem to be further need for extrusion once the plants have become diploid.

TYPES WITH SIXTEEN CHROMOSOMES

A small culture of plants (no. 6610) grown from seed sent by Mr. A. O. GARRETT from Utah, from a plant identified at the United States National Herbarium as *R. pyrifera* Rydb., exhibits a considerable amount of variation. All have glabrous leaflets as in *R. woodsii*. One plant has sepals that are erect on the fruit; another has sepals that are spreading or reflexed, with small lateral pinnae. The fruit of the latter resembles that of *R. californica*, and it also ripens later than that of the former plant. In 1927 very few flowers were produced, and, unfortunately, buds from two plants were fixed together. Cytological examination revealed that one plant is a regular diploid while the other has an extra pair of smaller chromo-

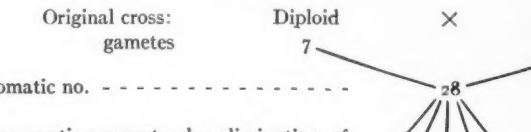
somes, giving eight pairs at diakinesis (figs. 22, 23). The bud with sixteen chromosomes also exhibits a phenomenon pertaining to nuclear disturbance which is absent in the diploid bud; that is, meiosis does not take place synchronously. Scattered in a single anther loculus some tetrads of young pollen grains can be found, as well as sporocytes undergoing reduction division and also some at diakinesis. This condition was noticed in the pollen sacs in the perfect flowers of *Populus tremuloides* (ERLANSON and HERMANN **20**); it is said by TÄCKHOLM (**59**) to characterize hybrid forms of *Rosa*. It is probable that the plants from Utah are descendants from a cross involving a diploid (perhaps *R. woodsii*) and some polyploid form (perhaps a tetraploid). In our culture 3753 a plant with fifteen chromosomes (plant 9/N1) and one with sixteen chromosomes (see later) have been discovered. All these aneuploid forms may have been produced from viable gametes that possessed eight instead of seven chromosomes. A hybrid plant with fifteen chromosomes might produce several gametes with eight chromosomes, and by self-fertilization give rise to offspring with sixteen chromosomes. It would also produce gametes with seven chromosomes and could therefore give progeny with fourteen and fifteen chomosomes, as shown in the following diagram:


Gamete of diploid parent - - -
7×14
Gamete of tetraploid parent
Triploid F1 - - - - - - - - -
21
Functional F1 gametes by elimination of univalents in meiosis
7 8 7 8
F2 somatic nos. - - - - - - -
14 15 16 14

It is significant that one of the pairs at diakinesis in the bud of *R. pyrifera* with sixteen chromosomes is smaller than the others. CLAUSEN (**9**) reports that in *Viola* hybrids in which the unpaired chromosomes divide at both meiotic divisions, the chromosomes that result are smaller than the others in the next generation.

Material fixed from plant no. 7/N37 in culture 3753 of *R. blanda glandulosa* showed no reduction stages in the microsporocytes. In one ovule, however, four young megaspores were found each showing eight chromosomes (fig. 25). The chalazal megaspore in the row is

already shrunken as if about to disintegrate; the two nearest the micropylar region are side by side instead of being in linear order, but one is larger than the other. TÄCKHOLM (**59**), and earlier STRASBURGER (**57**), found that in *Rosa* the micropylar megaspore is the functional one. Somatic counts of this plant show clearly sixteen chromosomes (fig. 26). It is conceivable that the plants of culture 3753, showing as they do fourteen, fifteen, and sixteen chromosomes, all arose from a fertile hybrid ancestor with fifteen chromosomes, suggested diagrammatically as follows:



Aneuploid forms are rare in *Rosa*. TÄCKHOLM only found five among 293 individuals; I found three among 108.

TRIPLOID TYPES

Among over 100 individuals which have been examined cytologically from the collection at Michigan, only two (and a possible third) have been found to be triploid, possessing twenty-one somatic chromosomes.

R. palustris×*carolina*, *no. 2872*

Specimens received from North Carolina as *R. lucida* (syn. *R. virginiana*) are vigorous and form a dense thicket. The stems are over 3 feet high, greenish brown, and weakly armed; they bear many flowers in small corymbs and begin to bloom in the second or third week of June at Ann Arbor. After anthesis all the hypanthia usually shrivel and fall off. Very rarely one or two small hips are formed, each containing only one or two achenes. The hypanthium in flower is glabrous; the pedicels bear a few stipitate hispid glands; the achenes are basally attached; and the sepals remain reflexed or spreading on the occasional hips.

A great deal of shrinkage and distortion due to fixation gave

similar effects to those noticed by CLAUSEN in his *Viola* hybrids, and impeded cytological examination. At diakinesis in the microsporocytes seven paired and seven unpaired chromosomes appear (fig. 27), the latter being noticeably small. All these elements become grouped on the equator at the first meiotic metaphase (fig. 28), and one member of each pair passes to each pole. The univalents become scattered along the spindle, and usually pass as whole chromosomes to the poles, being distributed at random. There is a tendency for the univalents to become fragmented at this stage, although this may be due to the fixing fluid. A similar effect was observed by GATES and THOMAS (**25**) in pollen mother cells of *Oenothera lata*, which is also a highly sterile form due to the presence of an extra unpaired chromosome. Lagging, unpaired chromosomes occasionally divide at the equator in the first anaphase; but only rarely, since, in the few sister interkinesis nuclei observed, in no case did the sum of their chromosomes total more than twenty-one. At late interkinesis and second metaphase varying numbers of chromosomes (usually 8–12) were found. The material does not show many cells in this stage, and only one case was observed in which the daughter nuclei contained either seven or fourteen chromosomes. Fig. 29 shows sister second metaphase plates with nine and twelve chromosomes respectively. Since no chromosomes are visible scattered in the cytoplasm, it is to be concluded that the unpaired seven passed whole to the poles, two going to one and five to the other. The second meiotic division exhibits several irregularities; extra spindles with two or three chromosomes are not infrequent. On the main spindles all the chromosomes usally begin to split and to separate simultaneously, but during anaphase some of the halves lag behind and fail to become included in the major grand-daughter nuclei. Apparently some of the chromosomes may fail to split at second anaphase and instead pass whole to the poles. Fig. 30 shows a second meta-anaphase spindle on which the seven large chromosomes (originally conjugants) have divided or are in the process of dividing; of the seven smaller chromosomes (originally univalents) three are dividing at the equator, one lies partly split at one pole, and the other three seem to be passing entire to the poles, two to one and one to the other. If all the chromosomes reach the poles,

the two resulting microspores will receive twelve chromosomes each, but in an entirely irregular manner. Polyspory is much in evidence. There are a few tetrads of pollen grains but usually from five to ten cells result from one sporocyte (fig. 31). In some microsporangia a few sporocytes fail to undergo meiosis, and can be observed full of fat globules, apparently breaking down among young microspores. Other sporocytes may give rise to two pollen grains only, and these may both be approximately of the same size and both large (fig. 32); rarely one member of a diad is almost twice the size of the other. It has not been possible to observe whether such diads are the result of a microsporocyte failing to develop beyond the first meiotic telophase, or whether at second telophase the chromosomes become included in two nuclei as observed by BLACKBURN and HARRISON (4) in *R. sabini*.

Culture 2872 is thus seen to be a good example of the "*Drosera*-type" of meiosis, and therefore resembles in its cytological behavior four of the five triploids whose meioses were observed by TÄCKHOLM. This type of meiosis is interpreted as being due to the fact that all the members of a smaller gametic chromosomal complement pair with members of a larger gametic complement, in the sporocytes of hybrids from a cross between two parents having different chromosome numbers. It is further characterized by TÄCKHOLM as having all the unpaired chromosomes irregularly distributed as whole chromosomes to the poles at first anaphase. It was originally observed by ROSENBERG (**47**) in his *Drosera rotundifolia*×*longifolia* hybrid, and since then in hybrids of *Morus* by OSAWA (**45**), of *Triticum* (SAX **51, 52**), of *Fragaria* (ICHIJIMA **33**), and of other plant genera. Complete accounts are given by TÄCKHOLM (**59**), SHARP (**55**), and MORGAN (**44**).

Since these *Rosa* plants came from North Carolina and their cytology reveals them to be offspring from a cross between a diploid (x=7) and a tetraploid (x=14), it is not difficult to assign putative parents. The only diploid species of the Cinnamoneae-Carolinae in the region is *Rosa palustris* Marsh., the tetraploid species being *R. carolina* L. and its varieties (or closely related and doubtfully distinct species), and possibly *R. virginiana* Mill. The triploid plants form dense thickets like those of *R. palustris*, although the habit is

more slender and weedy. The leaves are narrow and lance-elliptic, with finer teeth than in *R. carolina* and its allies, or than in *R. virginiana*. It flowers with the two latter species, two weeks or more before *R. palustris*. The occurrence of true *R. virginiana* in this region is doubtful, so that it is highly probable that these plants are hybrid *R. palustris*×*carolina*, the *R. humilis gracilis* of Porter MS according to RYDBERG (**49**). Our plants have weak, straight, infrastipular pickles or none, and the stems are bristly at the base. The fact that the hypanthia are glabrous is not significant, since the characteristic hispid glands are not infrequently absent from some individuals in both *R. palustris* and *R. carolina*.

Pollen examination revealed very little pollen in a flower bud, and of what was produced not a single grain was observed which was plump and yellow; all the grains were transparent and wrinkled, 60 per cent of them being misshapen and the pollen 100 per cent bad. In examining ovules from these plants no stages of meiosis have been observed, nor any embryo sacs. Some tetrads of megaspores have been seen, and in some cases all four spores appeared to be disintegrating. In one case the megaspore nearest the micropyle was still large while the other three were shrunken.

Culture 5590 (?) R. palustris×*virginiana*.—In a small culture of *R. palustris* grown from seeds sent by Dr. H. M. DENSLOW from New Jersey, six seedlings out of nineteen were noticed to have gland-compound teeth during the first season. In the following year (1926) all had simple teeth. They flowered for the first time in 1927, one plant beginning to bloom 8–10 days before the others. Unfortunately it was not observed until it was almost past flowering, and buds fixed then showed neither meiosis nor mitosis. However, a great deal of shriveled pollen was evident, and a count of mature pollen showed only 3 per cent of good grains. After flowering all the hypanthia shriveled and fell. In one bud 88 stamens were counted, about 100 less than the usual number in *R. palustris*. The plant is low in stature and the leaflets are distinctly ovate with coarse teeth; the other plants in the culture are typical of *R. palustris*. This sterile plant is therefore suspected of being a triploid hybrid, perhaps *R. palustris*×*virginiana*. This case emphasizes the necessity for growing large cultures of wild roses in order that the sterile and often

weak hybrid forms with interesting chromosome complements may appear, as well as to give an adequate idea of variation. It is often very difficult to obtain cultures because of slow and poor germination.

Culture 2949 (*?*) *R. blanda*×*carolina.*—The second plant which has been demonstrated cytologically to be a triploid with twenty-one somatic chromosomes was sent from Ithaca, N.Y., by Mr. W. E. MANNING. It is a low weak plant about 18 inches high, and produces very little vegetative growth. It is almost unarmed, possessing only a few weak paired infrastipular prickles. The leaflets, usually seven, are glabrous and glaucous, or have a few hairs on the veins beneath. The teeth are fine and ovate, smaller than is usual in *R. blanda* or *R. carolina*. It flowers toward the end of June, at the same time as *R. carolina*, bearing solitary flowers on the old wood but small terminal corymbs on some of the season's turions. Subglobose hips about 1 cm. in diameter are set. These and the pedicels are entirely glabrous. After anthesis the sepals are reflexed or spreading but are persistent. The achenes are basally attached, as is usual in *R. carolina* and occasional in *R. blanda*. The stamens vary from 140 to 155 per flower, about as in *R. carolina;* in fact, the plant strongly resembles some forms of *R. carolina* which are almost unarmed and eglandular.

At diakinesis in the microsporocytes of this triploid plant some cells show seven pairs of conjugating chromosomes, although fewer usually appear (fig. 33), and in a few cells the affinity is so weak that all twenty-one appear as distinct units, although some are grouped together in pairs they are not closely appressed. There is some extrusion of chromosomes at this stage, but so far as observed the number that pass from one cell into the next is variable, from one or two to all the chromosomes.

At early first metaphase all the bivalent and univalent members become grouped somewhat irregularly on the equator, the members of the pairs beginning immediately to disjoin and pass to opposite poles. The univalents split and seven half chromosomes often pass to each pole after the seven whole ones (fig. 34). There does not seem to be any definite grouping of the univalents and bivalents on the first metaphase plate, as is the case in many of the Caninae; in

fact, it is usual for some of the chromosomes to lie at various levels on the spindle, giving an irregular effect. Some of the univalents seem to have difficulty in dividing, so that the chromatic material becomes drawn out along the spindle fiber between the two halves. In some cells a few of the univalents fail to split, passing to one of the poles entire at first anaphase. In one such cell observed, nine chromosomes could be counted at one pole while four univalents were to be found dividing at the equatorial region; in another there were also nine chromosomes at one pole and five univalents were dividing at the equator. The distribution of chromosomes in 100 second metaphase plates was counted, and is shown as follows:

No. of chromosomes	7	8	9	10	11	12	13	14
No. of plates	1	4	14	23	15	13	12	18

The following distribution was observed in seventeen pairs of sister second metaphase plates. One pair had 14 and 13 chromosomes respectively; one had 14 and 12; two had 14 and 10; one had 14 and 9; one had 14 and 8; one had 14 and 7; one had 13 and 13; three had 13 and 12; two had 13 and 9; one had 12 and 11; one had 12 and 10; one had 11 and 10; and one had 10 and 10. In only five cases out of these seventeen does the sum of the chromosomes in two sister nuclei amount to only twenty-one or less, which might mean that in those cells none of the univalents had divided at first anaphase, providing all the chromosomes reached the poles. If all the univalents divided at first anaphase the daughter nuclei should each receive fourteen chromosomes. The two factors which prevent the sums of the chromosomes of sister nuclei from being twenty-eight are: first, all the univalents do not divide in every cell at first anaphase; second, all the split univalents are not included in the daughter nuclei. Nevertheless 30 per cent of these receive thirteen or fourteen chromosomes (fig. 35), and only in one case were sister plates observed containing seven and fourteen chromosomes respectively.

The second anaphase is usually initiated very regularly, all the chromosomes lying in one plane on the equator and all beginning to split simultaneously, in contrast with the conditions found in 2872. By the time second telophase is reached, however, some of the

chromosomes may lag behind the rest and fail to reach the poles. Many of the microsporocytes give rise to tetrads of microspores, and, although polyspory is frequent there are seldom more than two or three subsidiary spores produced. Examination of the pollen showed more shriveled grains than was expected, only 7.3 per cent of large plump grains being found in over 400 counted.

This plant (2949) resembles cytologically the triploid *R. centifolia major* examined by TÄCKHOLM (as no. 194), and the percentage of good pollen is very little more than that found in *R. centifolia major* by HURST (30). This type of meiosis has been called the "*Rosa*-type" by TÄCKHOLM, who first found it fully developed in the microsporocytes of the Caninae wherein all the unpaired chromosomes split at first meiotic anaphase. Our triploid from New York approaches very nearly to the *Rosa* type of meiosis, since it appears that nearly all the unpaired chromosomes divide at both meiotic divisions. The triploid from North Carolina, on the other hand, behaves almost as ROSENBERG'S *Drosera* hybrids, only rarely any of the unpaired chromosomes dividing at first anaphase.

Possible occurrence of fertile triploids

The outstanding point of interest about the New York triploid type is the fact that it produces a crop of good hips and achenes. As all students of *Rosa* know, the cytological examination of meiosis in the megasporocyte is difficult. Since our plants are small it has been possible to fix only a few buds; flowers have been needed for herbarium vouchers and also for seed. In ovules of accession no. 2949 good normal embryo sacs have been seen, as well as one case of two ovules in one carpel and a case of two embryo sacs in one nucellus. Such abnormalities have been observed before in the genus (STRASBURGER, TÄCKHOLM), and are considered by some to be characteristic of hybrids (JEFFREY 35, 36). No stages of meiosis have yet been discovered in the ovule, and three castrated flowers gave no fruit. In the absence of direct evidence as to chromosomal behavior during megasporogenesis, there are two alternate possibilities to be considered as explanations for the fertility of this plant.

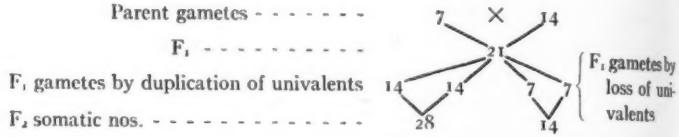
As a first alternative the plant may resemble the Caninae, in which case all the unpaired chromosomes in the megasporocyte pass

as a group to one pole at first meiotic anaphase, and the daughter nucleus receiving fourteen chromosomes gives rise to the functional megaspore. If, as in the Caninae, only those pollen grains containing seven chromosomes are functional, there would be only a small chance for this plant ever to produce zygotes by self-pollination, if one judges from the distribution of chromosomes in second meiotic metaphase nuclei. By the time second telophase is reached some grand-daughter nuclei may receive only seven chromosomes, due to lagging during the second anaphase, as is the case in the Caninae. Should the specimen correspond in this way to the roses of the Caninae, it would thereby give rise to a freely fruiting triploid race of North American roses, either by apomixis or by the fusion of a fourteen-chromosomed egg with a seven-chromosomed sperm nucleus. The extreme rarity of triploids in my material, and the fact that none have been reported by others from this continent, would seem to preclude this possibility, although our plant might be the starting point for such a line. Such triploids, strongly resembling tetraploid types, as does our plant, and setting fruit freely, would be very difficult to recognize as hybrids in the field unless one examined the pollen.

The second alternative is that meiotic behavior in the ovules of this plant corresponds to the process in the microsporocytes, as is the case in other Cinnamomeae. If this were so we could expect an appreciable number of both megaspores and microspores to receive fourteen chromosomes, and there would be produced a zygotic tetraploid form with twenty-eight chromosomes from a triploid parent by self-pollination. The plant would then resemble CLAUSEN'S *Viola* hybrid, in which the somatic number in the F_2 generation was increased over that in the F_1 by a double splitting of unpaired chromosomes during meiosis. KARPECHENKO (**37**) also found that this took place in some *Brassica*×*Raphanus* hybrids. The increased chromosome number observed by BREMER (**8**) in a hybrid from a species cross in *Saccharum* may have come about in this way. The possibility that our plant may produce some spores that receive only seven chromosomes is not ruled out.

In the central United States there are many *Rosa* forms which are very puzzling taxonomically. Some of them appear to be inter-

mediate between *R. suffulta* (tetraploid) and *R. blanda* (diploid), for example, *R. relicta* Erlanson. It may be that these actually originated from crosses between diploid *R. blanda* and tetraploid forms, and that the resulting triploid hybrids were fertile as is 2949, giving rise to fertile tetraploids or perhaps to some diploids in the following manner:



According to HURST's theory of differential septets, if the two parents were respectively CC and CCDD, there would be the re-establishment of the tetraploid parent type by the duplication of the unpaired D chromosomes in the F_1 hybrid. To a certain extent this is true, since the rose forms in question have many characteristics of other tetraploids, yet they are distinct from *R. suffulta* and exhibit some characteristics of *R. blanda*.

The tetraploid *R. damascena* is known to have given rise to a triploid form in cultivation, and HURST (31) believes this to be due to the loss of a septet by extrusion. Since our fertile triploid strongly resembles *R. carolina*, it might be attributed to a loss of seven chromosomes in one of the parent gametes, but the idea of its possible hybrid origin seems to be more in conformity with known proceedings in other organisms.

In 1927 six field-pollinated hips were collected from 2949, containing 13, 8, 6, 5, and 4 achenes respectively. Out of three buds which were self-pollinated, one contained only two achenes and the other none. Too much emphasis must not be laid on the apparent failure of self-pollination, since it has been found very difficult to obtain seeds in this way from many of the North American *Rosa* species, particularly in *R. blanda*. It may be that bagging the whole inflorescence causes the pollen to become damp and non-functional, or there is the possibility of some self-sterility. FOCKE (23) gives *R. blanda*×*lucida* as a garden hybrid.

Infrequency of triploid roses

The fact that I have been able to find so few triploid roses is surprising, especially considering the great number of triploid forms LONGLEY (**41, 42**) found in *Rubus* and *Crataegus*. One reason for this no doubt is the fact that our tetraploid and diploid species of *Rosa* are so often separated both in space and in time of flowering. In the eastern part of the continent *R. blanda* (diploid) flowers before *R. virginiana*, *R. carolina*, and their allies (tetraploids), while *R. palustris* (diploid) comes into flower last of all. In the western part *R. woodsii* and its allies (diploid) flower before *R. suffulta* (tetraploid) and before *R. californica* (tetraploid). Also, the diploid forms are characteristic of waterways and swamps, while the tetraploids are usually found on dry uplands and plains. Further extensive field work will no doubt bring to light other hybrids between the two groups, and crosses already have been successfully made experimentally.

TETRAPLOID TYPES

There are two very distinct groups of tetraploid rose species in North America, widely separated geographically. These are the *R. californica* group on the Pacific Coast, and the large group comprising *R. virginiana*, *R. carolina*, and *R. arkansana* and their allies. The former two are distributed over the eastern half of the continent north to the Great Lakes and the St. Lawrence, and the latter is characteristic of the great plains of the Mississippi drainage system.

R. californica and allied species.—TÄCKHOLM (**59**) found *R. californica* to be diploid with seven pairs at diakinesis. I have examined one individual of this species and one of each of the closely related species *R. brachycarpa* Rydb. and *R. aldersonii* Greene, which are perhaps only varieties of *R. californica*. All three show fourteen pairs of chromosomes at reduction division in the pollen mother cells (figs. 38–40). The allied species *R. myriantha* Carr, obtained from Nevada City, which morphologically is somewhat intermediate between *R. californica* and *R. pisocarpa*, is also tetraploid (fig. 41).

Middle-western and eastern species.—Cytological examination has confirmed TÄCKHOLM'S findings that *R. virginiana* Mill., *R. carolina* L. (*R. humilis* Mill.), and *R. suffulta* Greene all have twenty-eight somatic chromosomes and fourteen pairs at reduction division.

This group of roses is extremely variable and difficult taxonomically. In addition to the usual morphological variations, this group shows marked geographical and local races which present great difficulties to descriptive definition. It has long been a moot point whether *R. carolina* is a variety of *R. virginiana* or not because of the intergrading series of forms found between the two in the northeastern United States (BEST 2). In the middle western region there is an equally notable intergrading series of forms between *R. carolina* and its varieties and the *R. arkansana-suffulta* group, giving forms like *R. rudiuscula* Greene and *R. conjuncta* Rydb.

Tetraploid and diploid races of R. subserrulata Rydb.—When a specimen from Oklahoma, which agrees morphologically with *R. subserrulata* Rydb., was examined cytologically, it was very unexpectedly found to be diploid, showing seven chromosomes at first and second metaphases with only a slight amount of lagging during meiosis, and an occasional chromosome off in the cytoplasm. Another specimen belonging to this species, from Arkansas, was examined and found to be tetraploid, as would be expected from its close resemblance to *R. carolina*. The tetraploid plant (no. 6072) shows several irregularities during meiosis. At first anaphase from three to five pairs may lag behind the others; fig. 59 shows three lagging pairs as well as two unpaired chromosomes, one of which is beginning to divide. Early first telophases usually show fourteen chromosomes (fig. 61), and in one case two distinct groups of seven chromosomes were found at this stage (fig. 60). Except for this example, which could be due to chance grouping, I have not seen any evidence for chromosomes separating in groups of seven at reduction division as HURST'S theory would seem to demand, if the septets, which he regards as linkage groups, are to remain intact. According to HURST'S theory the diploid *R. subserrulata* might be regarded as having been produced from a tetraploid form by loss of a diploid set of septets. The origin of both the diploid and tetraploid types of *R. subserrulata* can also be postulated from a fertile triploid producing functional gametes with both seven and fourteen chromosomes, as suggested under the New York specimen 2949.

This case of *R. subserrulata* is one of only two in which have been found different chromosome numbers in two individuals apparently

belonging to the same species. The diploid *R. subserrulata* was received from Mr. RALPH SHREVE late in 1926, and flowered in the cold frame in 1927. At present I do not know whether it produces flowering turions or not. The common widespread glandular form of *R. carolina*, *R. serrulata* Raf. (*R. carolina* var. *glandulosa* Farwell), was found to have twenty-eight somatic chromosomes in a Michigan specimen (fig. 47) and fourteen pairs at diakinesis in a specimen from Maryland (no. 5566, plant A).

Meiosis in megasporocyte.—The diad stage of meiosis was observed in an ovule of a plant from Aurora, Illinois (no. 3501) which I consider to be a form of *R. rudiuscula* Greene (fig. 62). One of the cells is in interkinesis while the other is entering second metaphase. Both show fourteen split chromosomes.

MEIOTIC IRREGULARITIES IN TETRAPLOIDS

Early stages of meiosis in the tetraploid forms resemble similar stages in the diploid types. The chromosomes are attached end to end, and each usually becomes paired with one adjoining individual. Condensation of the chromosomes is irregular. Fig. 38 shows a diakinesis nucleus in *R. californica* in which there are twelve pairs, a ring of three and one long, unpaired chromosome. Rings of four chromosomes are often present during diakinesis, but they usually break up into two pairs by the time the first meiotic metaphase is reached, and well condensed groups of four are not as common as are rings (figs. 42, 52). There is most frequently only one ring of four to a nucleus, although two or even three have been observed (fig. 51). Fig. 46 shows a cut cell at early first anaphase in which a ring of four chromosomes has not yet succeeded in condensing and reaching the spindle.

In some nuclei at diakinesis there seems to be a tendency for half of the chromosomes to pair intimately quite early, while the other fourteen lie adjacent to each other in pairs but seem to have a decidedly weaker affinity. This is particularly noticeable in species of the *R. suffulta* complex (figs. 49, 53). Some of the members of this group sometimes possess certain characteristics of *R. blanda*, and are often associated with it in the field; and, as has been suggested, may have arisen as a cross between that species and some

tetraploid. The discovery of the fertile triploid 2949, which could conceivably give rise to a fertile race of tetraploids by self-pollination, suggests a possible origin for such anomalous tetraploid forms. One would expect that chromosomes which were originally halves of a single individual would pair very intimately at diakinesis.

In some cases a few of the chromosomes in a tetraploid microsporocyte fail to pair at all at diakinesis, and, just as among some diploids, they appear on the first metaphase spindle as univalents (fig. 56). During first anaphase there is nearly always some lagging of one or two pairs, but each pole usually receives fourteen (figs. 40, 43). It is also evident that occasionally some of the chromosomes that fail to pair will divide at late first anaphase (figs. 54, 57, 59). This was observed by TÄCKHOLM in his tetraploid specimen no. 132, which was named *R. blanda*, as well as in some of his tetraploid hybrids. Second anaphase may be regular (fig. 50), or there may be some lagging and extra nuclei at second telophase (fig. 58).

Cytologically nearly every tetraploid specimen examined showed meiotic irregularities which differ only in degree from those found in known tetraploid hybrids examined by TÄCKHOLM. A dwarf plant of *R. subglauca* Rydb. from Alberta (no. 3492) shows many such irregularities. At first meiotic metaphase nearly all the chromosomes lie loosely associated in pairs (fig. 55) or some fail to pair. Fig. 56 shows four univalents on the spindle. Some of them split at first anaphase (fig. 57), and there is some lagging so that all do not reach the poles. Fig. 58 shows a sporocyte at second telophase in which one pair of grand-daughter nuclei contain fourteen chromosomes each; the other pair twelve and thirteen respectively. Five extra chromosomes and two split ones lie in various positions about the cell, making a possibility of nine chromosomes not included in the four nuclei. Since this makes a total of sixty-two chromosomes instead of a normal fifty-six, three univalents have divided at both metaphases.

Polyspory is more frequent among tetraploids (fig. 48) than among diploids, and the percentage of bad pollen is larger on the average (table IV). Extrusion is frequent, as in the diploids, and the number of chromosomes thus lost seems to be arbitrary.

Possible origin of tetraploid types

One of the most striking characteristics of meiosis in the tetraploids is the presence of groups and rings of four (instead of two) chromosomes at diakinesis. This is shown in several of the figures. Täckholm does not seem to have observed it. It is frequent in my material and resembles the configurations found in tetraploid *Datura* plants by Belling and Blakeslee (1), although in no case has a cell been observed that is completely quadrivalent in synapsis. The tetraploid condition may have arisen in these forms by duplication from the diploid, and have become modified in later generations by crossing-over, non-disjunction, and hybridization, until a single individual only possesses one to three quadrivalent sets of homologous chromosomes. Tetraploid roses may have arisen more than once on this continent by duplication in more or less sterile diploid hybrids. This is known to have occurred in the case of *Primula kewensis* (Digby **17**), in *Nicotiana* (Clausen and Goodspeed **10**), in *Raphanus*×*Brassica* hybrids (Karpechenko **37**), and in *Rosa wilsoni* (Harrison and Blackburn **5**). Winge (**66**) believes that lack of harmony between chromosomes from two parents, even when their numbers are the same, may lead to reduplication, giving tetraploids from hybrid diploids. From his work on *Viola*, Clausen (**9**) concludes that such doubling is important in biological evolution, and will produce "biologically stable types." Duplication, on the other hand, which produces true tetraploidy with quadrivalent sets of homologous chromosomes, he believes to be of little consequence in phylogeny, such types being unstable and likely to revert to the original diploid state.

The possibility that a tetraploid form with all the chromosomes pairing might be produced from a cross between a diploid and a hexaploid among the Cinnamomeae should not be entirely overlooked, even though this would involve pairing between the chromosomes from one parent, a condition that has not been observed in *Rosa*. Longley (**43**) found that in a cross between *Vaccinium corymbosum* (haploid number of 24) and *V. virgatum* (with $x=36$), the F_1 hybrid occasionally showed as many as thirty pairs at diakinesis, although the hybrid was highly sterile.

MORPHOLOGICAL CHARACTERISTICS AND DISTRIBUTION OF TETRAPLOIDS

It soon became noticeable among our living collection that nearly all the North American tetraploid species are characterized by the ability to produce flowers terminally in corymbs on the season's turions, a behavior peculiarly adapted to a prairie habitat where the aerial stems are likely to be frozen down in winter and burned off by fire in summer. They are also characterized by their long flowering period, some of them continuing to bloom after ripe fruit is developed, a performance never observed in any of the other native groups, although exhibited by *R. rugosa* Thunb. *R. acicularis*, however, will occasionally have a second blooming late in a mild autumn.

I had hoped that the ability to flower on the season's turions would prove to be a criterion for tetraploidy in American roses, and for the most part this is true. In *R. virginiana*, the largest of the eastern tetraploid species, this ability is only weakly developed and some plants will not produce flowering turions every year. It is very common in *R. californica*. *R. ratonensis* Erlanson in some ways resembles *R. woodsii*, but can immediately be distinguished from that species by its strong turions bearing terminal corymbs. It is a tetraploid.

The most unexpected exception to this generalization was the discovery of a diploid specimen of *R. subserrulata*, a possible hybrid origin of which has been previously considered.

Another exception to this character being confined to tetraploids is *R. foliolosa* Nutt., a diploid species of the Carolinae from Texas and Oklahoma. This is a low semi-herbaceous species that bears very little wood over 2 years old. It flowers in small corymbs, terminally on the annual shoots. If this species originated as a haploid from a tetraploid form and still retained the tetraploid "ever-blooming" characteristic, it would from its southern distribution be an argument in support of HURST's theory of the loss of extra septets of chromosomes from polyploids in southern habitats. It is a very distinct species, its narrow numerous leaflets and small (sometimes curved) prickles resembling, if anything, diploid *R. palustris*, whose western limit is well to the east of the range of *R. foliolosa* (text fig. 1), rather than any form of tetraploid *R. carolina*.

Our collection does not contain any mature *R. nitida* Willd. This species was reported as diploid by TÄCKHOLM, and it may also possess the ability to flower on the season's turions, although this is not mentioned in descriptions and can seldom be discovered from herbarium specimens. It will be of interest to discover whether the other dwarf species, *R. gamella* Willd. and *R. nanella* Rydb., also Carolinae with limited ranges in the northeastern Atlantic region, are diploid or tetraploid. *R. relicta* Erlanson, a dwarf form from Illinois, and the low growing *R. bushii* Rydb. are both tetraploid. The third exception is the case of a dwarf plant from Calgary, Alberta (no. 3571). After three years in the garden it is little more than a foot high, a small branched woody bush resembling in habit the specimen of *R. subglauca* from Alberta (no. 3492). The former plant agrees with the description of *R. alcea* Greene. It grows very slowly and has produced no turions. The dwarf *R. subglauca* has produced one or two flowering turions. Both these specimens are tetraploid.

With the exception of *R. virginiana*, in the northeast, and of *R. californica*, the North American tetraploid roses are of low stature, usually under a meter. The tetraploid species belonging to the section Cinnamomeae are more specialized in habit and inflorescence type than the related diploid forms, and are perhaps of more recent origin. The setaceous stems and numerous leaflets are considered by BOULENGER (7) as relatively primitive characteristics in *Rosa*, but they almost always appear on young turions, and would be expected on plants with a semiherbaceous habit. Some of these forms, more particularly *R. suffulta* and its allies in the Mississippi region, have a markedly semiherbaceous habit, which appearing in a frutescent group is generally considered a more highly evolved condition (SINNOTT and BAILEY 56). BEWS (3) points out that the stream bank and swamp habitat is one of the primitive unchanging habitats of angiosperms, and that the grassland habitat is a relatively recent one; hence he maintains that plants adapted to the latter are derived and not primitive forms. As already stated, the North American diploid roses are characteristic of waterways (as are the hexaploids), while the tetraploids belong to the prairies and dry uplands.

From every point of view the tetraploid forms seem to be more

highly evolved and of more recent origin than any other form in the group of Cinnamomeae-Carolinae in America.

Among the tetraploid Carolinae, *R. virginiana* stands out as the most robust and fruticose type. This species seems to be confined to the northeastern Atlantic region, although a whole series of forms can be found between it and the typical low weedy *R. carolina*, as well as between this latter species and the tetraploid Cinnamomeae of the *R. suffulta* complex. All these types flower almost synchronously when grown together. The significance of this will be considered in the discussion of phenology in *Rosa*. The tetraploid Carolinae have a very extensive north and south range, from the St. Lawrence to northern Mexico. It is noticeable that the glandular types of the *R. carolina* complex preponderate in the southern part of its range; this is also true for the western diploid *R. woodsii* complex, which has an even greater north and south range than the Carolinae, stretching from British Columbia to Chihuahua.

Hexaploid types

All North American forms of *R. acicularis* examined, except one individual from Alaska, showed twenty-one pairs of chromosomes at metaphase at the first meiotic division in the microsporocytes (fig. 63). This species is typically boreal and alpine, and Schneider (54) was without doubt in error in attributing a Texan specimen to it. Specimens collected in the Sangre de Cristo Mountains of southern Colorado by C. O. Erlanson grew at altitudes between 8000 and 13,000 feet; they are hexaploid and agree with Rydberg's descriptions for *R. butleri* and *R. underwoodii* (fig. 64). After having examined several thousand herbarium specimens of North American roses, I have seen no specimens belonging to the *R. acicularis* complex from farther south than the mountains of southern Colorado.

As has been found by others, *R. nutkana* Presl. is also hexaploid, as are the allied species *R. spaldingii* Crép. (fig. 65) and *R. macdougali* Holz. *R. engelmanni* S. Wats., which is somewhat intermediate between *R. acicularis* var. *sayiana* and *R. nutkana* in the northern Rockies, is also hexaploid. The hexaploid forms exhibit very few irregularities in meiosis. Both divisions are usually almost schematic in regularity. Extrusion occurs (fig. 64); delayed pairing at di-

akinesis and lagging chromosomes have only been observed in one or two cases; polyspory is rare (fig. 66); and pollen examination reveals a very low percentage of poor pollen, in all cases less than 10 and often under 5 per cent.[6]

The *R. acicularis* forms are the first of the American species to flower in the spring, and the pollen mother cells are frequently damaged by frost at the end of May. They are followed in about a week by *R. nutkana* and its allies. In all these species the buds are relatively large (8–10 mm. long) when pollen formation takes place. Although these forms show few cytological disturbances, it is evident from the variations in cultures that they hybridize freely among themselves. Neither is artificial hybridization between hexaploid forms difficult. The hexaploids are not larger plants than the diploid Cinnamomeae.

Just as in the region of the Great Lakes, botanists have always found difficulty in distinguishing some forms of *R. blanda* from *R. acicularis;* so in the Rocky Mountain region there are many forms intermediate between *R. nutkana* and *R. acicularis*. In the case of the two latter species the flowering times are more nearly approximate, and the chromosome complements of the two species are alike, thus making chances for successful crossing greater. I suspect that *R. engelmanni* is a product of such a cross, and hope to produce it artificially in the garden. Hexaploid species have been crossed successfully to diploid forms. Crossing tetraploids with hexaploids has not been attempted, for, although their ranges overlap in the eastern foothills of the Rocky Mountains and in the northern great plains region, the wide difference in time of flowering between the two groups almost precludes this cross in the field.

As HARRISON and BLACKBURN (**27**) have pointed out, the hexaploid condition probably arose from the union of a tetraploid with a diploid gamete followed by duplication in a triploid hybrid. The greatest concentration of hexaploid types on this continent seems to be in the northern Rocky Mountain region, and their distribution is continuous in all directions from thence. No hexaploids have been

[6] I wish to express my thanks to Dr. REHDER and Mr. JACK for extending to me the privilege of collecting cytological and herbarium material from the interesting hexaploid roses in the Arnold Arboretum collection in 1926.

found outside the continuous range of *R. acicularis* in regions where diploid and tetraploid forms both occur; that is, in the northwestern part of the great plains, and in the United States east of the Mississippi (text figs. 1, 2); so that it would appear as though the hexaploid type has not arisen more than once, if at all on the American continent. There has not been discovered a hexaploid in the section Carolinae. The hexaploid types of Cinnamomeae may have entered America from northeastern Asia and spread south and east over the continent.

Meiosis in ovule.—Meiosis has not been observed in the megasporocyte of any hexaploid type. Stages in fertilization as well as a normal two-celled embryo have been observed in *R. acicularis* var. *lacorum* from northern Michigan (text fig. 3). Castrated buds have continually failed to give any fruit.

R. nutkana and related species.—The diploid *R. pisocarpa* and the tetraploid *R. californica* both show some morphological resemblance to *R. nutkana*. This fact has been evident to students of the Pacific Coast roses. WATSON (63) placed *R. californica* and *R. pisocarpa* in the same subdivision. In determining TÄCKHOLM'S material, ALMQUIST identified the diploid "*R. californica*" specimens from Lund and Kew as varieties of *R. nutkana*, and gave the same treatment to the *R. pisocarpa* specimens. It is fairly certain that former students of the Pacific Coast roses have usually had only small series of specimens at their disposal, and have misunderstood the species as did CRÉPIN (RYDBERG 48). Nevertheless there is a superficial morphological resemblance between these species in some respects, just as there is between *R. acicularis*, *R. blanda*, and *R. suffulta* in the northern great plains region, although one would never confuse them in a living collection. There is thus some evidence supporting the suggestion of HARRISON and BLACKBURN (27) that the North American Cinnamomeae species have a dual origin, one group of species arising from *R. nutkana*. Their "neat proof," however, from the presence of a specific rose gall insect is unfortunately not justified by the work of KINSEY (40) on which they base it.[7] It has

[7] Dr. KINSEY informed me that it was not his intention to affirm that *Diplolepis bassetti* is "confined to *R. nutkana*." The following are extracts from his letters in 1926: "The host record which I gave for *Diplolepis bassetti* should have been applied only to the type material of variety *bassetti*. . . . . I have no determinations of any sort

FIG. 1.—Map showing distribution of Carolinae: area of diploid species delimited by dots; tetraploids by crosses

seemed to me for some time that the two series of species groups (1) *R. nutkana* (hexaploid), *R. pisocarpa* (diploid), *R. californica* (tetraploid), in the far west and (2) *R. acicularis* (*R. sayi*) (hexaploid), *R. blanda* (diploid), *R. suffulta* (tetraploid), or *R. acicularis*, *R.*

FIG. 2.—Map showing distribution of American Cinnamomeae: area of hexaploid species delimited by crosses; diploids by dots; tetraploids by wavy line.

woodsii, *R. arkansana* in the Mississippi region, are analogous in the origin and relationship of the contained forms. If the chromosome numbers in our species have become reduced in phylogeny, as HURST claims, this has apparently not been in a simple descending series, and there seems to be no reason to suppose that the process which

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on the roses from which I secured additional material of the same variety. . . . . The only material which I have shows nothing but a remnant of a stem without leaves. . . . . I have no notion what rose this species (*Diplolepis bassetti*) occurs on and I am very much inclined to believe that it occurs on more than one rose in this wide range (i.e. Oregon, Idaho and Utah)."

produces forms related to the polyploid species but with smaller chromosome numbers is anything other than hybridization, followed by the loss of unpaired chromosomes, as previously suggested under diploid species.

Octoploid type

A large bush of *R. acicularis* from Europe at the Arnold Arboretum, which was grown from seed sent from the Royal Gardens at Kew, has only twenty-one pairs at diakinesis, as found also by Pen-

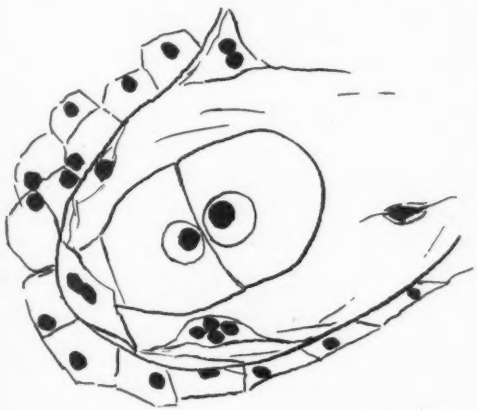


Fig. 3.—Two-celled embryo of *R. acicularis* var. *lacorum* no. 6007 (hexaploid)

land (46), and not twenty-eight as in European material from Sweden and Russia examined by Täckholm.

An individual from a collection sent by Professor C. H. Morgan from Fairbanks, Alaska (accession no. 6447) was found to be an octoploid with twenty-eight pairs at diakinesis (figs. 67, 68). Meiosis in this form is regular throughout and no polyspory has been observed. The plant possesses very glandular foliage and narrow pendant hips, and fits well into *R. acicularis* var. *lacorum* as described from the Great Lakes region (Erlanson 18), differing from that form only in its earlier flowering period. Herbarium specimens of the European forms of *R. acicularis* were examined in the Crépin Herbarium at Brussels in 1925, and no distinguishing morphological character could be found, that would hold out in a series of speci-

mens, to distinguish them from North American types of the species. All that can be said is that glands on the foliage are far commoner in American than in European types, in which glands are exceptional. There does seem to be some difference in habit, however, the European bushes that I have seen in botanical gardens branching more freely and reaching a greater height than our specimens. The Alaskan plants in our collection thus far show no marked habit differences from the other American forms. This, therefore, is the second case in which two individuals have been discovered apparently belonging to the same species yet possessing different chromosome numbers (the other case is mentioned under the tetraploid *R. subserrulata*).

The octoploid *R. acicularis* has exceptionally few deformed pollen grains. In 400 grains examined not one shrunken and transparent grain was observed, the only irregularity exhibited in the pollen being 3.75 per cent of distorted and angular (though plump) grains amongst the normal ones.

Polyspory and apomixis

Pollen examination

The first published report of pollen examination in American rose species is that of Crépin (15), who reports "pollen pur" in all the forms examined, except in the case of *R. californica*, the pollen of which he found "tantôt complètement pur, tantôt mélengé d'environs $\frac{1}{3}$ de grains atrophiés." He thought originally that the presence of dwarf grains gave "une précieuse indication dans le cas où une forme est soupçonnée d'hybridité," but was constrained to modify his views on account of the pollen sterility present in the Caninae. He found that the amount of bad pollen varied in different flowers from the same bush, and probably also from year to year. Cole (12) reported shriveled grains among the pollen of several American rose species but did not give actual counts. Harrison and Blackburn (27) showed that the amount of bad pollen among roses of the Caninae varies from 10 to 100 per cent.

In order to examine pollen, anthers were taken from buds just before the petals opened, and stored over calcium chloride in a desiccator. The pollen was examined dry under the microscope and

the percentage of shriveled grains in a sample of about 500 was calculated. As shown in table IV, the octoploid *R. acicularis* and all

TABLE IV

PERCENTAGE OF STERILE GRAINS IN POLLEN OF AMERICAN ROSES

SPECIES	ACCESSION NO.	ORIGIN	PERCENTAGE STERILE POLLEN
Diploid			
R. gymnocarpa	4006	Idaho	9
R. ultramontana	5292	Washington	4.7
R. salicetorum	3530	Idaho	38.0
R. woodsii	5560	Montana	9.0
R. fendleri	2938	Colorado	25.6
R. blanda	2895	Iowa	10.0
R. blanda	3502	Illinois	52.0
R. blanda var. hispida	2681	N. Dakota	4.3
R. blanda var. hermanni	2686	Michigan	6.0
R. blanda var. glandulosa	3753/9	Michigan	17.6
R. blanda var. glandulosa	5/N10	Michigan	25.0
R. schuetteana	5891	Michigan	8.3
R. michiganensis	5890	Michigan	3.6
R. palustris	4412	Michigan	10.0
R. palustris	5714	Michigan	4.8
R. palustris	8626	Ohio	9.5
Tetraploid			
R. californica	5849	California	26.0
R. aldersonii	5367	California	22.0
R. suffulta	2682	N. Dakota	15.5
R. suffulta	3001	Kansas	29.0
R. rudiuscula	3501	Illinois	25.8
R. bushii	3080	Missouri	26.0
R. relecta	8320	Illinois	56, 77
R. carolina	5716	Michigan	25.4
R. carolina	2946	New York	9.5
R. virginiana	6071	Maine	24.4
R. virginiana	4651	Connecticut	10.0
Hexaploid			
R. spaldingii	5298	Washington	8.0
R. spaldingii	5549	Washington	8.6
R. butleri	8271	Colorado	0.0 (8.3% angular grains)
R. underwoodii	8270	Colorado	8.3
R. engelmanni	3721	Wyoming	10.8
R. acicularis var. lacorum	6008	Michigan	8.4
Octoploid			
R. acicularis	6447	Alaska	0.0 (3.6% angular grains)
Triploid			
R. sp. indet.	2872	N. Carolina	100.0
R. sp. indet.	5590	Connecticut	97.0
R. sp. indet.	2949	New York	92.7

hexaploid forms examined had about 10 per cent or less of shriveled pollen. Several diploid forms have less than 10 per cent of shriveled grains. *R. salicetorum* and *R. fendleri* showed 38 and 25.6 per cent

respectively. There is great variation in the amount of sterile pollen in different individuals of *R. blanda* and its varieties, varying from 4.3 to 52 per cent. Most of the tetraploids show from 15 to 25 per cent of bad pollen. Considerable difference is shown between two individuals of the same species in *R. carolina* and in *R. virginiana*. *R. relicta* Erlanson has the highest amount of sterile pollen of any form examined (except the triploids), one bud having 56 and another 77 per cent of bad pollen.

These counts demonstrate that many of our native rose species are partially sterile, a characteristic often associated with hybridity. It is very interesting to find that, among forms with all pairing chromosomes, the higher polyploids show the least amount of sterile pollen, while the tetraploids show the most; the diploids occupy an intermediate position in this respect, showing much variation.

Castration experiments

Täckholm (**59**) has given an account of the castration experiments carried out in Europe on *Rosa*. Harrison and Blackburn (**27**) report that several of the English Caninae are facultatively apomictical. Hurst (**30**) reported the production of normal achenes after castration in *R. gallica* and other garden forms. Castration has been carried out on several North American species during the past three years, and in only two cases were fruits obtained subsequently. These were both in 1927 on plants of *R. blanda* from northeastern Illinois; plant 8321 on which two buds were castrated gave one small hip containing two achenes; six buds were castrated on plant no. 3502 and every one set a plump hip filled with achenes. In castrating, the androecium and the stigmas were removed with a razor blade but the buds were not bagged. The case of 8321 may have been due to a failure to remove all the stigmas; the case of 3502 is significant when its high percentage (52 per cent) of bad pollen is considered. Ernst (**21**) maintains that polyspory and apomixis are consequent upon hybridization.

Phenology in relation to phylogeny

Taxonomists formerly seem to have agreed that *R. acicularis* is the most primitive member of the Cinnamomeae (Boulenger **7**), and my observations tend to confirm this. The tetraploid forms

seem to be the most highly evolved and specialized of the American Cinnamomeae, while the related diploids are intermediate between the hexaploids and tetraploids. There appear to be two distinct series of hexaploid, diploid, and tetraploid forms, with ranges on the western and eastern sides of the Rocky Mountains respectively. In each series the hexaploids are boreal and alpine in distribution, the tetraploids temperate and southern (text fig. 2).[8] In both of these series the diploids occupy a middle position in the southern part of the range of the series as a whole, and are intermediate in flowering time between the early-flowering hexaploid species and the later-flowering tetraploids.

There is some evidence that the phenological procession in the flora of the northern hemisphere, taken by and large, gives some clue to phylogenetic relationships, the vernal forms being the most primitive, as suggested by HARSHBERGER (**28**) and maintained by ILLITSCHEVSKY (**34**) at the International Congress of Botany in 1926 at Ithaca. This principle may also help in an understanding of the phylogeny of the species in some large boreal genera.

Phenological notes have been taken on the University of Michigan rose collection for three years, and each species complex is found to have a specific and characteristic flowering period. When taken in relation to each other each group of forms comes into flower in a definite order. In fact the "flowering time" of any individual is often a very good specific character, a fact to which CRÉPIN (**14**) first called attention. A collection of plants belonging to *R. blanda* var. *hispida* which was received from Manitoba (no. 5771) contained one plant which did not differ markedly from the others in habit, yet it began to bloom about 10 days later. Cytological examination revealed that the earlier blooming form was diploid while the latter one was tetraploid. Upon closer examination the latter specimen was found to have 7–9 glabrous leaflets; the petals were pale salmon

[8] In text figs. 1, 2, and 4 the distribution areas have been plotted whenever possible from specimens seen by the writer. An "!" means that a specimen has been verified from that particular region. Doubt about the occurrence of a certain group is indicated by an "?" immediately following the number, which denotes the chromosome constitution of the group. The limits of the areas of distribution are approximately correct, although they may have to be extended in some directions when more specimens have been studied.

color, not rose pink as in the other plants; and the hips were larger with a slight neck. I have placed it under *R. arkansana* Porter. Although we have several collections of each species complex from different parts of its range, a hexaploid type from the mountains of Montana flowers synchronously with hexaploid forms from the mountains of southern Colorado and from central Michigan; even as tetraploid forms from North Dakota and Alberta bloom no earlier than those from Missouri. The many varieties of *R. blanda* obtained from stations all over its range all come into flower during the same week. The octoploid *R. acicularis* from Alaska is the first of the American plants to come into flower; in 1926 it bloomed a week before any of the hexaploid forms of this species.

Possible origin of polyploid series

CINNAMOMEAE

The conclusion that hybridization between many of our wild forms is general and of common occurrence in nature is supported by: (1) the successful production of good achenes by artificial crossing of different species with the same chromosome number, of diploids with hexaploids and of diploid with tetraploid forms; (2) the marked variation in the progeny obtained from seeds taken from individual wild plants in the field; (3) the large and variable percentage of sterile pollen present in many wild individuals; (4) the meiotic irregularities similar to those found by TÄCKHOLM in plants with a balanced number of chromosomes but which were known to be hybrids. Furthermore, it appears as though all crosses between parents with unlike chromosome number are not sterile. The evidence is strong for the theory of HARRISON and BLACKBURN of "the hybrid origin of orthoploid series in *Rosa* paralleling that in other plant genera." If this premise be accepted, it is possible to postulate an origin for the polyploid series which we find in the North American Cinnamomeae (table V). As COCKERELL (11) pointed out, the octoploid and hexaploid types were probably built up by duplication from primitive diploid roses, and although HURST does not mention this in his preliminary paper (31), this is also his idea of their origin.

The northern limit of hexaploid types on this continent is at present unknown, as is the southern limit of the octoploid *R. acicularis*. It is reasonable to suppose that both types reached America

TABLE V

Phylogenetic diagram

AMERICAN CINNAMOMEAE

Ancestral diploid forms by duplication and hybridization → higher polyploids

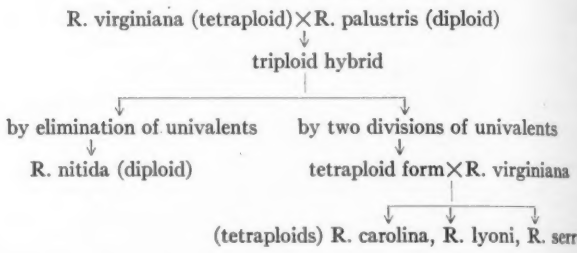
R. acicularis (octoploid) × R. nutkana (hexaploid)
↓ (or related form)
Heptaploid hybrid
↓
Later generations by elimination of univalent chromosomes
↓ ↓ ↓
R. engelmanni, R. acicularis sayiana, R. acicularis bourgeauiana
(hexaploid types)

R. nutkana (hexaploid) × (diploid) Generalized ancestral type of R. woodsii group
↓
Tetraploid hybrid
7 bivalents and 14 univalents at diakinesis
↓
Later generations by elimination of univalents
↓
R. pisocarpa (diploid) × diploid form
↓
diploid hybrid
↓
by duplication
↓
R. californica (tetraploid)

R. acicularis sayiana (hexa.) × R. woodsii ancestor (diploid)
↓
Tetraploid hybrid
↓
Later generations by elimination of univalents
↓ ↓
R. blanda (dipl.) × R. macounii, R. fendleri (diploids)
↓
diploid hybrid
↓
by duplication
↓ ↓
R. suffulta, R. arkansana (tetraploid) × R. blanda
↓
triploid hybrid
↓
by two divisions of univalents
↓
R. relicta (tetraploid)

TABLE V—*Continued*

DIAGRAM OF POSSIBLE ORIGIN OF SOME CAROLINAE


R. virginiana (tetraploid)×R. palustris (diploid)
triploid hybrid
by elimination of univalents
by two divisions of univalents
R. nitida (diploid)
tetraploid form×R. virginiana
(tetraploids) R. carolina, R. lyoni, R. serrulata

from the northwest, coming from northeastern Asia, as suggested for the hexaploids by HARRISON and BLACKBURN (**27**).

In 1927, pollen from the octoploid *R. acicularis* was collected May 24 and kept in a desiccator until June 14; it was then used to pollinate four buds of *R. nutkana* from Idaho. Large hips were set, containing 16, 17, 22, and 22 achenes respectively. Seedlings from this cross would be expected to be heptaploid with forty-nine somatic chromosomes, and to show twenty-one pairs and seven univalent chromosomes at diakinesis. In F_2 and later generations a fertile hexaploid race might be reestablished by the loss of the unpaired chromosomes by lagging at meiosis, as has been found to occur in wheat hybrids (SAX **51, 52, 53**; KIHARA **38, 39**). These hexaploid descendants should possess some of the characteristics of each parent. Such a cross may well have been the origin of the American hexaploid forms such as *R. engelmanni*, *R. acicularis* var. *sayiana*, and *R. acicularis* var. *bourgeauiana*.

A good yield of achenes was obtained by crossing *R. woodsii* (diploid) with *R. spaldingii* (hexaploid), and also from crossing *R. salicetorum* (diploid) with *R. nutkana* (hexaploid). Some such cross, followed by an elimination of unpaired chromosomes in subsequent generations, may have given rise to the diploid *R. pisocarpa*. Some related sterile diploid form, by duplication, may have originated the tetraploid *R. californica*. Such a phylogeny does not seem too far-fetched, and would account for the resemblances already noted between *R. nutkana*, *R. pisocarpa*, and *R. californica*.

A hexaploid *R. acicularis* crossing with some member of the

diploid *R. woodsii* group, by elimination of unpaired chromosomes in later generations, could be imagined to have produced the *R. macounii-fendleri* group of diploid forms. Some one of these, crossing with *R. blanda* (or other diploid), may have resulted in a sterile diploid form, which by duplication gave rise to a fertile tetraploid race, the *R. suffulta-arkansana* group, which latter group of forms always seemed to CRÉPIN to be a variety of *R. blanda*.

The distribution map of the American Cinnamomeae (text fig. 2), although only approximate, brings out the fact that the diploid and hexaploid forms occur in that area of the continent which was occupied by the Pleistocene ice sheet (COLEMAN 13), the diploid forms having spread appreciably south of this area only in the western mountainous regions and in the foothills. The distribution area of *R. californica* lies well to the south of the glaciated area, and that of the tetraploid *R. arkansana* group is, for the most part, also to the south of it. The latter group is spreading rapidly both northward and eastward, however, having been found in the last few years both in southern Michigan and in northern New York.

The general area of distribution of *R. blanda* (text fig. 4) contrasts with that of the other Cinnamomeae in being northeastern and in not reaching the Rocky Mountains, although many closely related forms are found there. Whether *R. blanda* was already on this continent before the Pleistocene ice sheets advanced, and whether it therefore, upon their final withdrawal, advanced northward and westward, finally meeting the hexaploid and diploid forms coming in from the extreme northwest, is an interesting speculation.

CAROLINAE

The generalized distribution map of the Carolinae (text fig. 1) shows that these forms occupy the southeastern part of the continent, chiefly that region not occupied by the Cinnamomeae. In the case of the Carolinae, the diploid and tetraploid types occupy almost the same area, the tetraploids going farther into the southwest. The Cinnamomeae and Carolinae overlap chiefly in the Great Lakes region and in the central part of the Mississippi Valley, where tetraploid forms belonging to both sections are found. It is somewhat certain that these tetraploids hybridize and give rise to intermediate

forms such as *R. rudiuscula* Greene, *R. bushii* Rydb., and *R. conjuncta* Rydb.

As stated elsewhere (ERLANSON 19), *R. palustris* and *R. blanda* appear to cross in the northern Lake Michigan region and to give stable forms. *R. virginiana* may be an ancient tetraploid type, and is typically limited to the northeastern Atlantic region. *R. palustris* crossed with *R. virginiana* would give a triploid hybrid, which would probably be sterile. Since no fertile hexaploid forms are known in this group, a fertile tetraploid may have been reestablished from such a sterile triploid by all the unpaired chromosomes dividing at both meiotic divisions, as in our accession no. 2949 from Ithaca, N.Y., and this tetraploid may have been the ancestral form of the complex of *R. carolina*, *R. lyoni*, *R. serrulata*. Specimens of *R. palustris* with gland-compound teeth have been observed in herbaria.

No sterile wild diploid type has yet been discovered, although there is the possibility that they exist and produce fruit apomictically.

The fact that vigorous forms, originally of hybrid origin, may spread beyond the distributional limit of at least one parent is seen in the case of *R. blanda* var. *glandulosa*, which is found farther south along the shores of Lake Michigan than *R. acicularis*. An alternate origin of the hexaploid forms on this continent from a cross between a tetraploid and diploid followed by duplication does not seem feasible from a consideration of the distribution data.

Hurst's theory of differential septets

Owing to the interest which HURST's theory of the origin of forms with lower numbers from higher polyploids in *Rosa* has aroused, the evidence for and against his interpretation, taken from findings in American material, follows.

Evidence favoring hypothesis

1. Octoploid *R. acicularis* is apparently arctic, with the hexaploid *R. acicularis* types boreal and alpine in distribution. The resemblance between the two types could be interpreted to mean that one "double septet" (fourteen homologous chromosomes) does not affect the octoploid plant and could therefore be dispensed with if eliminated by extrusion.

2. Two forms occur in southern stations, with flowering times and habit characteristic of tetraploids, yet having only fourteen chromosomes. These are *R. foliolosa* and the diploid *R. subserrulata*. The latter appears to be almost identical morphologically with the tetraploid *R. subserrulata*, and both it and *R. foliolosa* seem to be "haploid tetraploids" rather than simply primitive diploids. If this were so these forms could be interpreted as having arisen from tetraploid ancestors by the loss of half of the chromosomes ("one double septet") in southern latitudes.

Evidence against hypothesis

1. Neither HARRISON and BLACKBURN nor I have been able to discover any convincing cytological evidence for distribution of the chromosomes in sets of seven during reduction division in *Rosa*, as would be required by HURST's theory if the "differential septets" are to remain together as compound linkage groups. On the contrary, the cytology of many American forms demonstrates the independent and often irregular distribution of chromosomes at meiosis. In the same plant varying numbers of bivalent groups are present in different cells at first metaphase, and varying numbers of the univalents may be split at first anaphase. The fact that all gametes resulting from irregularities in meiosis, or containing extra chromosomes, are not sterile is shown by the existence of fertile, vigorous aneuploid plants.

2. Chromosome extrusion is frequently observed in the microsporocytes of *Rosa*, but the number of chromosomes thus lost from the cell seems to be arbitrary and variable, and is probably due to disturbances consequent upon hybridization.

3. It is very doubtful that all crosses between different "simple septet" species (diploids) give sterile diploid hybrids. Intermediate types between *R. palustris* and *R. blanda* (which are surely different "simple diploid species") in northern Michigan, such as *R. schuetteana* and *R. michiganensis* (ERLANSON 19), have been found to be diploid and have very little sterile pollen.

4. If there are races within some species with different chromosome complements which are indistinguishable morphologically, as seems to be the case in *R. acicularis* and *R. subserrulata*, then we

cannot base a classification of the genus on the "numbers of somatic septets of chromosomes present in the species," as HURST proposes to do.

Summary

1. In this study 107 wild roses from known stations in North America were examined cytologically. The majority of the specimens fall into the three classes, diploid, tetraploid, and hexaploid, seven being the basic number. One specimen was octoploid, two were triploid, and three aneuploid with the somatic number not a multiple of seven.

2. Measurements show that the cells are larger in the polyploid forms.

3. The diploid American roses are robust plants, larger as a rule than the related polyploid species. Many of them exhibit irregularities during meiosis. In some individuals incomplete pairing at diakinesis, lagging chromosomes, and polyspory are frequent; these are believed to be spontaneous hybrids produced by the crossing of different species, or the descendants of such hybrids.

4. It is concluded that crossing occurs in the field between different diploid species in *Rosa*, giving rise to at least partially fertile diploid offspring.

5. Three fertile aneuploid plants, two with sixteen somatic chromosomes and one with fifteen, have been found among cultures of diploid plants. One such culture exhibits a great deal of variation and appears to be a later generation of a cross between the diploid *R. blanda* and the hexaploid *R. acicularis* of Michigan. Such a cross would give, in the first generation, tetraploid hybrids with seven pairs and fourteen unpaired chromosomes. During meiosis in these hybrids the unpaired chromosomes probably lag on the spindle, giving some fertile gametes with only seven or eight chromosomes, which by self-fertilization give rise to fertile diploid or aneuploid plants.

6. Two triploid plants have been examined cytologically. Both have seven paired and seven unpaired chromosomes at first reduction division. One exhibits the "*Drosera*-type" of meiosis and is sterile. The other has the "*Rosa*-type" of meiosis, in which the seven univalent chromosomes divide twice, making it possible for

some spores to receive fourteen chromosomes, although this is prevented in many cells by lagging on the spindle. This latter plant is not apomictical yet it sets good fruit. Reduction in the megasporocyte of the fertile triploid has not been observed. The plant may be a starting point for a race of triploid roses analogous cytologically to the pentaploid Caninae, or it is able to give either tetraploid or diploid offspring by self-fertilization. A third plant with over 90 per cent of the pollen sterile is suspected of being a triploid hybrid from *R. palustris*×*R. virginiana*.

7. *R. californica* and related species were found all to be tetraploid with fourteen pairs at diakinesis.

8. The tetraploid species examined cytologically nearly all exhibit irregularities during meiosis and also polyspory. They thus resemble hybrid forms in their cytological behavior.

9. Both tetraploid and diploid races morphologically similar were found in *R. subserrulata* Rydb.

10. The tetraploid forms often exhibit some rings of four chromosomes at diakinesis, but none have been found that are completely quadrivalent. It is concluded that they originated by duplication of diploid hybrids and have hybridized with each other.

11. The tetraploids are characterized by being able to flower on the season's turions and by a long flowering period. Many of them are semi-herbaceous or dwarf, and are believed to be recent or derived forms adapted to prairie and dry upland habitats.

12. *R. nutkana* and related species, and all but one of the specimens of *R. acicularis* and its varieties, were found to be hexaploid, with twenty-one pairs at diakinesis.

13. Hexaploids exhibit few meiotic irregularities.

14. All hexaploids in America apparently occupy one continuous range, and it is thought that they have not originated on this continent from a sterile triploid hybrid by duplication, but that they migrated from Asia as hexaploids spreading southward and eastward.

15. There are two parallel series of American Cinnamomeae, each consisting of hexaploid, diploid, and tetraploid types: (1) on the Pacific Coast *R. nutkana*, *R. pisocarpa*, and *R. californica;* (2) in the Mississippi Valley *R. acicularis*, *R. blanda*, and *R. suffulta*.

16. A specimen of *R. acicularis* from Alaska was found to be octoploid with twenty-eight pairs at diakinesis. The plant does not differ markedly from the hexaploid variety *lacorum*, but is distinguished by coming into flower earlier. It has pure pollen.

17. Examination showed that the hexaploids have a small proportion (under 10 per cent) of sterile pollen. Some diploid specimens have almost entirely good pollen while others have 10–15 per cent sterile grains. Most of the tetraploids showed 15–30 per cent sterile pollen, although individuals of *R. carolina* and of *R. virginiana* were found with 90 per cent of their pollen good. One tetraploid had 56–77 per cent of the pollen bad.

18. Good fruit was obtained after castration in one case only, that of a specimen of *R. blanda* (diploid) from Illinois which had 52 per cent bad pollen.

19. The flowering times of each of the large species groups are specific and characteristic when taken in relation to each other. The phenology of the species of American Cinnamomeae seems to coincide with phylogenetic relationship, those coming into flower first being the most primitive. The flowering order is: octoploids, hexaploids, diploids, tetraploids.

20. Cytological and morphological evidence support the theory of HARRISON and BLACKBURN of the hybrid origin of orthoploid series in *Rosa*. A possible origin of the North American polyploid series is worked out from this premise.

21. The presence of octoploid and hexaploid races of *R. acicularis* in America, and also of diploid and tetraploid races of *R. subserrulata*, is suggested as evidence in support of HURST'S theory.

22. Cytological observations on American roses do not support HURST'S theory of differential septets in *Rosa* which would require that sets of seven chromosomes separate together during meiosis. Extrusion is thought to be due partly to imperfect fixation and partly to disturbances consequent upon hybridization.

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EXPLANATION OF PLATES XVI–XIX

Except as otherwise stated, all figures were drawn with a Zeiss compensating ocular no. 20 and a 1.5 mm. apochromatic objective, with the aid of a camera lucida. They were reduced two-thirds in reproduction.

PLATE XVI

FIG. 1.—*R. bracteata* 3676, diploid; first metaphase plate, seven pairs.

FIG. 2.—*R. gymnocarpa* 4006, diploid; first meta-anaphase, seven pairs.

FIG. 3.—*R. pisocarpa* 4634, diploid; diakinesis showing three types of pairing.

FIG. 4.—*R. woodsii* 8152, diploid; early first telophase.

FIG. 5.—*R. salicetorum* 3530, diploid; early diakinesis with chain of six chromosomes.

FIG. 6.—*R. granulifera* 5925, diploid; two cells at diakinesis each with six pairs and two unpaired chromosomes, exhibiting distortion of nuclei and extrusion of chromosomes.

FIG. 7.—*R. hypoleuca* 8154, diploid; diakinesis, multipolar spindle.

FIG. 8.—*R. blanda* 3942, diploid; second metaphase plates.

FIG. 9.—*R. blanda* var. *hispida* 5771 (plant B), diploid; first meta-anaphase spindle with one pair off in cytoplasm.

FIG. 10.—*R. blanda* var. *glandulosa* 3753 (plant 5/N3), diploid; somatic prophase.

FIGS. 11, 12.—*R. blanda* var. *glandulosa* 3753 (plant 2/N38), diploid; diakinesis nuclei with two unpaired chromosomes and rings of two chromosomes.

FIG. 13.—Somatic prophase in root tip of semihardy, sterile segregate of culture 3753; fourteen chromosomes.

FIG. 14.—Fifteen chromosomes segregate in culture 3753 (plant 9/N1); somatic prophase in parietal cell of pollen sac.

FIG. 15.—Plant 9/N1, culture 3753, diakinesis; six pairs and three unpaired chromosomes; majority of chromosomes from contiguous cell in cytoplasm.

FIG. 16.—Culture 3753, plant 9/N1, first metaphase; seven pairs and one univalent chromosome.

FIG. 17.—Culture 3753, plant 9/N1, first metaphase; four bivalents and one univalent on spindle, three bivalents in cytoplasm.

FIG. 18.—Culture 3753, plant 9/N1, sister second metaphases with seven and eight chromosomes.

FIG. 19.—Culture 3753, plant 9/N1, sister second metaphases each with seven chromosomes; extra chromosome (?) on surface of shrunken protoplast.

PLATE XVII

FIG. 20.—*R. acicularioides* 8261, diploid; first metaphase with five bivalents and four univalent chromosomes.

FIG. 21.—*R. acicularioides* 8261, diploid; somatic prophase in integument.

FIG. 22.—*R. pyrifera* 6610, aneuploid; diakinesis, seven bivalent and two univalent chromosomes.

FIG. 23.—*R. pyrifera* 6610, aneuploid; diakinesis, seven pairs and one smaller eighth pair.

FIG. 24.—*R. woodsii* 4477, five young microspores produced from one sporocyte (ocular no. 10, objective 1.5 mm.).

FIG. 25.—Culture 3753, plant 7/N37, four megaspores (two superimposed) each with eight chromosomes, surrounded by archeosporium (ocular no. 10, objective 1.5 mm.).

FIG. 26.—Culture 3753, plant 7/N37, sixteen chromosomes in somatic prophase in cell from chalazal region.

FIG. 27.—Culture 2872, triploid; entering first metaphase, seven bivalent and seven univalent chromosomes.

FIG. 28.—Culture 2872, triploid; first metaphase, seven bivalent and seven univalent chromosomes.

FIG. 29.—Culture 2872, triploid; sister second metaphase plates with twelve and nine chromosomes.

FIG. 30.—Culture 2872, triploid; second anaphase spindle with fourteen large chromosomes (the halves of seven original bivalents) and eight others. This nucleus received seven univalents at first telophase, one of these has split, two are dividing longitudinally at equator, one lies at one pole partly split.

FIGS. 31, 32.—Culture 2872, sterile triploid; products of two microsporocytes ten cells from one and two large cells from the other (ocular no. 10, objective 1.5 mm.).

FIG. 33.—Culture 2949, triploid; twenty-one chromosomes at diakinesis, including four or five pairs.

FIG. 34.—Culture 2949, fertile triploid; late first anaphase, seven chromosomes at each pole (originally all paired), seven univalents dividing at equator.

FIG. 35.—Culture 2949, triploid; sister second metaphase plates, with fourteen and nine chromosomes (ocular no. 20, objective 1.8 mm.).

FIG. 36.—Culture 2949, triploid; somatic prophase in nucellus.

FIG. 37.—Culture 2949, triploid; somatic prophase in stylar cell, twenty-one chromosomes.

PLATE XVIII

FIGS. 38*A*, 38*B*.—*R. californica* 5849, tetraploid; cut cell at diakinesis, twelve bivalent chromosomes, ring of three and one uncondensed chromosome.

FIG. 39.—*R. brachycarpa* 5301, tetraploid; diakinesis, twelve pairs and a ring of four.

FIG. 40.—*R. aldersonii* 5367, tetraploid; sister second metaphase plates with fourteen chromosomes each.

FIG. 41.—*R. myriantha* 3537/B, tetraploid; fourteen pairs at diakinesis.

FIG. 42.—*R. obovata* 2651, tetraploid; late diakinesis, fourteen pairs (note condensed group of four).

FIG. 43.—*R. lyoni* 2883/A, tetraploid; late interkinesis, sister nuclei each with fourteen chromosomes.

FIG. 44.—*R. carolina* 5808/B, tetraploid; late first anaphase, two pairs lagging.

FIG. 45.—*R. carolina* 5808, ring of four chromosomes from cut cell at diakinesis stage.

FIG. 46.—*R. carolina* 5808, cut cell at first metaphase, ring of four and pair of chromosomes off in cytoplasm.

FIG. 47.—*R. serrulata* (*R. carolina* var. *glandulosa*) 3938, tetraploid; somatic prophase in stylar cell with twenty-eight chromosomes.

FIG. 48.—*R. carolina* var. *litoralis* 2654; seven cells the product of one microsporocyte (ocular no. 10, objective 1.5 mm.).

FIG. 49.—*R. rudiuscula* 4001, tetraploid; diakinesis, twelve pairs and one group of four; seven pairs with chromosomes more closely appressed and condensed.

FIG. 50.—*R. bushii* 3080, tetraploid; second anaphase, each grand-daughter nucleus receiving fourteen chromosomes.

FIG. 51.—*R. suffulta* 2682, tetraploid; two cells at diakinesis: one (cut) showing two rings of four, two unpaired chromosomes and seven pairs; other with eleven pairs and two unpaired chromosomes being extruded into next cell and two pairs left.

FIG. 52.—*R. suffulta* 2692, tetraploid; late diakinesis showing fourteen pairs, including condensed group of four chromosomes.

FIG. 53.—*R. suffulta* 5705, tetraploid; diakinesis with fourteen chromosomes fairly closely paired, chain of six, group of four, and four chromosomes loosely grouped in pairs.

FIG. 54.—*R. suffulta* var. *valida* 4459/A, tetraploid; late first anaphase, two pairs lagging much behind the others.

PLATE XIX

FIG. 55.—*R. subglauca* 3492, tetraploid; first metaphase plate with fourteen pairs.

FIG. 56.—*R. subglauca* 3492, tetraploid; first metaphase with twelve bivalent and four univalent chromosomes.

FIG. 57.—*R. subglauca* 3492, tetraploid; end of first anaphase, showing irregular distribution of chromosomes: twelve at one pole, one near equator, fifteen (including one split) at opposite pole, one small chromosome in cytoplasm (perhaps due to a univalent having divided at first anaphase).

FIG. 58.—*R. subglauca* 3492, tetraploid; second telophase, one spindle with thirteen chromosomes at each pole and one dividing at equator, the other with thirteen at one pole and twelve at other and one lagging chromosome; also one

split chromosome and four others off in cytoplasm. Extra chromosomes resulting from some univalents dividing at both anaphases.

FIG. 59.—*R. subserrulata* 6072, tetraploid; late first anaphase, ten chromosomes at each pole, three pairs and two univalents at equator, one of the univalents dividing.

FIG. 60.—*R. subserrulata* 6072, tetraploid; first telophase with two groups of seven chromosomes.

FIG. 61.—*R. subserrulata* 6072, tetraploid; first telophase with fourteen chromosomes.

FIG. 62.—*R. rudiuscula* 3501, tetraploid; meiosis in megasporocyte, sister nuclei showing fourteen chromosomes, one in interkinesis and other entering second metaphase.

FIG. 63.—*R. acicularis* var. *sayiana* 3754, hexaploid; early diakinesis, twenty-one pairs, four groups of two pairs.

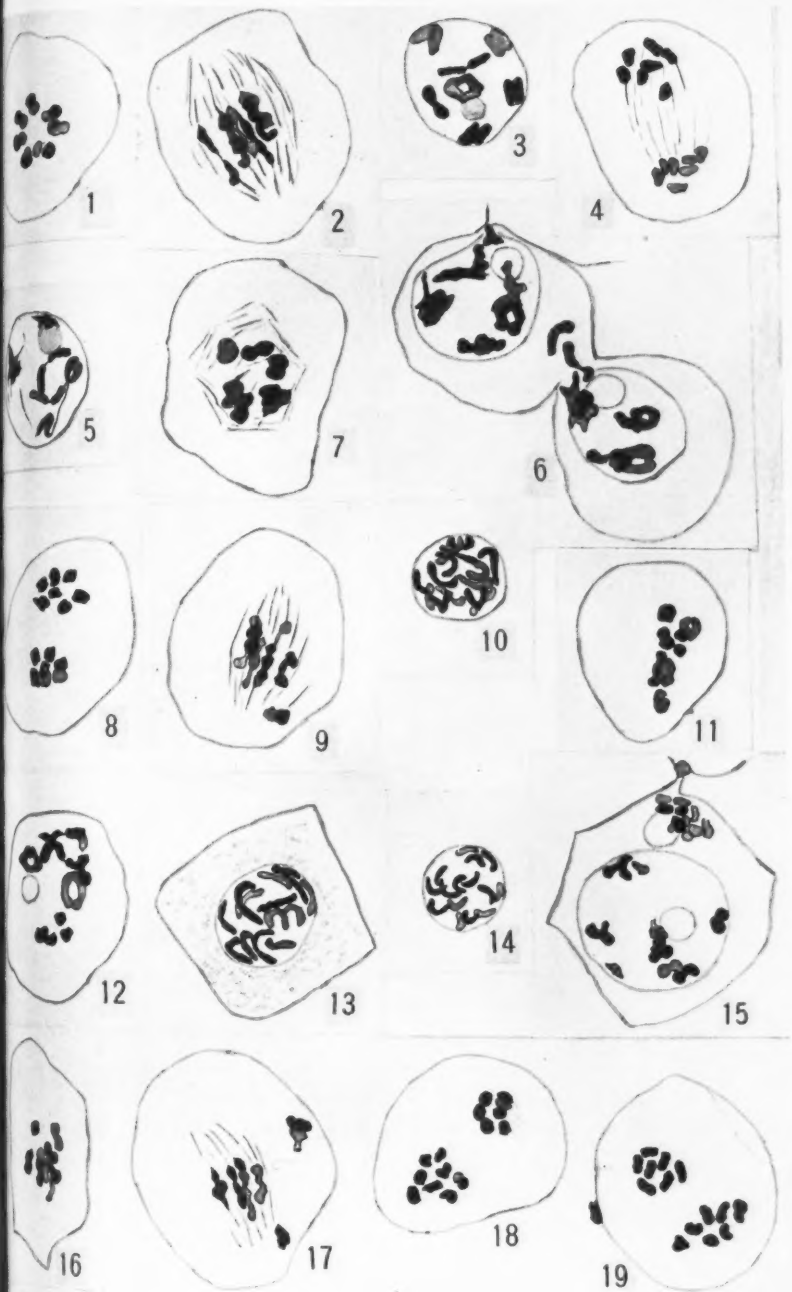
FIG. 64.—*R. underwoodii* 8270, hexaploid; early diakinesis with sixteen pairs in main and five pairs in subsidiary nucleus.

FIG. 65.—*R. spaldingii* E10, hexaploid; first metaphase plate with twenty-one pairs.

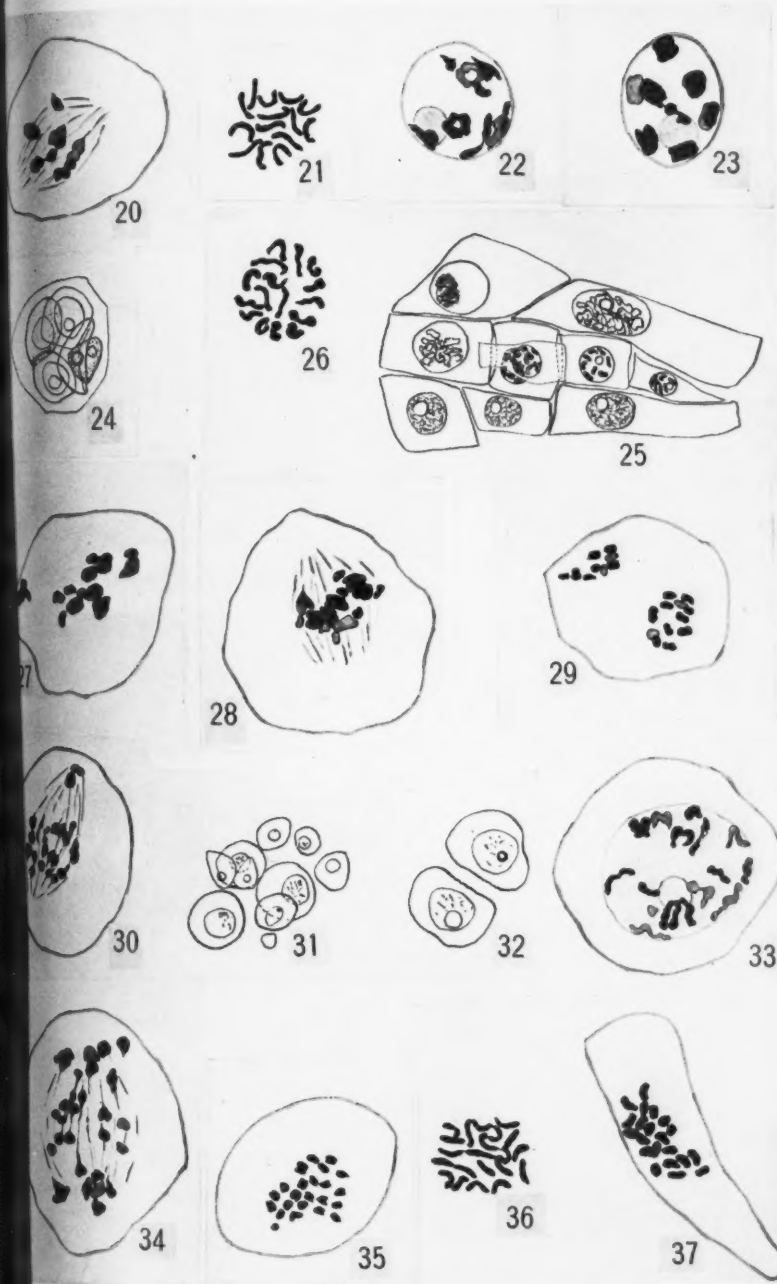
FIG. 66.—*R. macdougali* E8, hexaploid; five nuclei produced from one microsporocyte.

FIG. 67.—*R. acicularis* 6447, octoploid; diakinesis with twenty-eight pairs (ocular no. 20 and 1.8 mm. objective).

FIG. 68.—*R. acicularis* 6447, octoploid; first metaphase plate with twenty-eight pairs.



ERLANSON on ROSA



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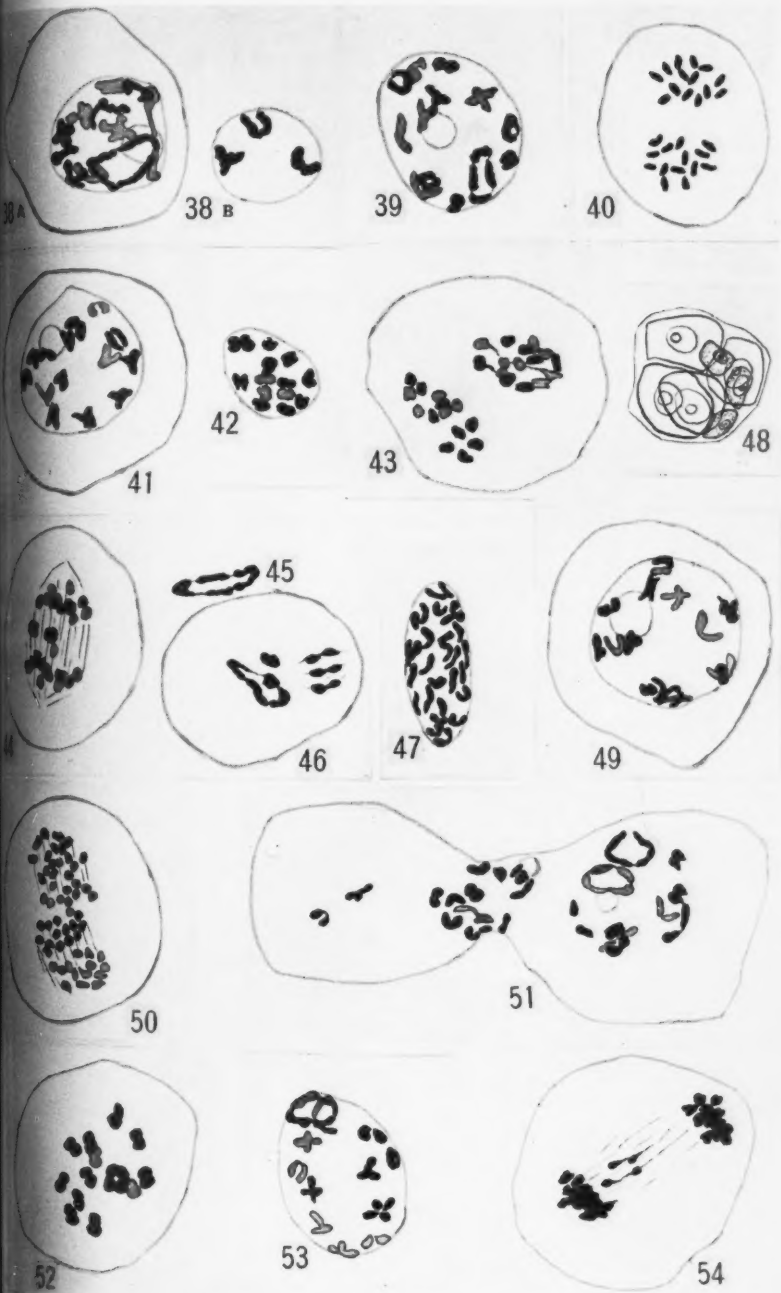

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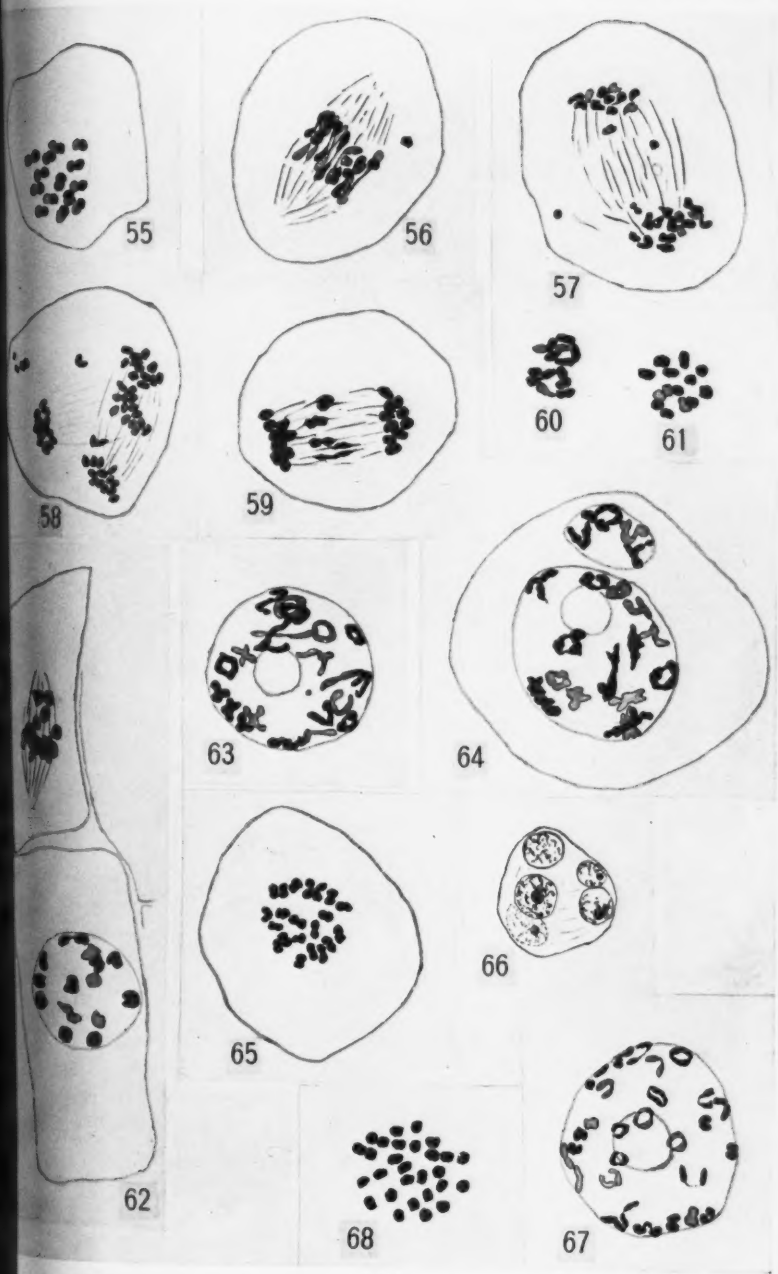

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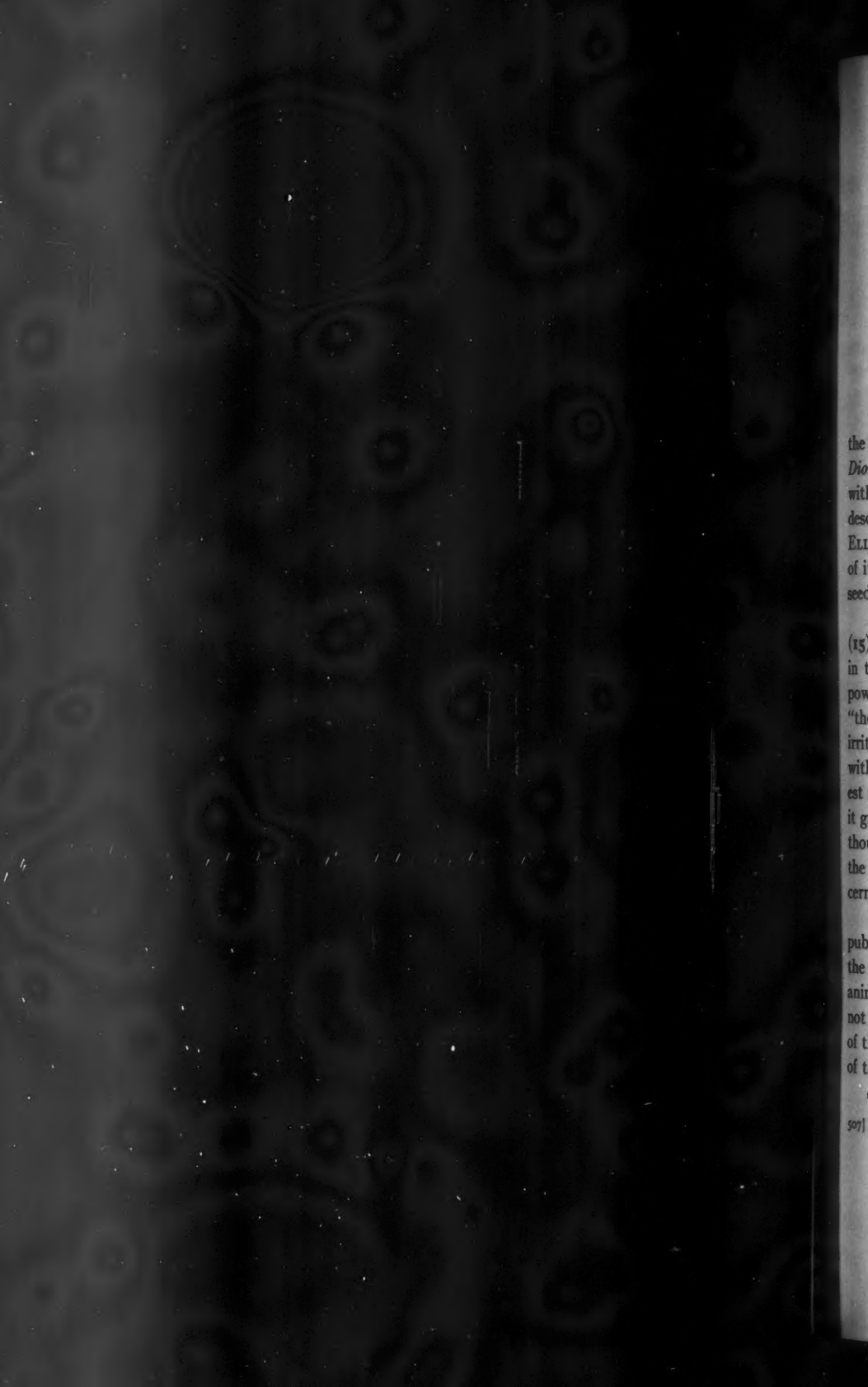


DEVELOPMENT OF DIONAEA MUSCIPULA

I. FLOWER AND SEED[1]

CORNELIA MARSCHALL SMITH

(WITH PLATES XX–XXIV AND THREE FIGURES)

Introduction

This paper presents the results of a somewhat detailed study of the flower and seed development of the sole known species of *Dionaea*. A great number of researches have been published dealing with this extremely interesting insectivorous plant, since its original description in a letter of September 23, 1768, to LINNAEUS by JOHN ELLIS (17), but not one of these papers embodies an adequate study of its flower and seed development, and of the germination of the seed.

Dionaea was introduced into Kew Garden by WILLIAM YOUNG (15) in 1768. From that time forward botanists have been interested in the plant, primarily, however, in the movement and digestive powers of the leaf. EDWARDS (15) called attention to the fact that "the small spines, mentioned and figured by ELLIS, are the only irritable points, and that any other part of the leaf may be touched with impunity." CURTIS (9) and CANBY (5) made some of the earliest recorded observations on the insectivorous habits of *Dionaea*, as it grew in its native habitat near Wilmington, North Carolina. Although OUDEMANS (30) examined the leaves histologically, and noted the absence of nyctitropic movements, his report was chiefly concerned with observations on the sensitivity of the leaves.

Between 1874 and 1877 numerous papers on this plant were published. SANDERSON (36) demonstrated the similarity between the contraction of a leaf of *Dionaea* and that of the muscle of an animal in response to electrical stimulation. In 1875, DARWIN (11) not only gave a brief description of the roots and the enlargement of the stem from which the roots spring, and a detailed description of the leaves, but also reported the results of many interesting ex-

[1] Botanical contribution from the Johns Hopkins University, no. 100.

periments which he performed on the latter. Further, he concluded that the closure of the leaf is due to the contraction of a layer of cells on its upper surface. BALFOUR (1) discussed irritability, contraction, secretion, digestion, and absorption in *Dionaea*. He suggested that the spiral thickenings and their elasticity might play an important rôle in the nature and cause of contraction. It was upon the investigations of the anatomy and physiology of the leaf made by KURTZ (26), that MUNK (28) based his work. Although the major portion of the latter's research is concerned with an attempt to explain the action of the leaf from an electrical standpoint, he points out the additional fact that the sensitive hair is divided into two parts, a lever and a base, and that the base alone is capable of receiving stimuli. The results obtained and reported by DARWIN from his experiments on the leaves of *Dionaea* served as an impetus for the anatomical investigations of the vegetative organs of the entire plant by FRAUSTADT (18). Two other papers dealing with the mechanism which produces the movement of the leaves of *Dionaea* were published during this period respectively by DE CANDOLLE (6) and BATALIN (2).

Both GOEBEL (19) and MACFARLANE (27) described and figured the sensitive hairs of *Dionaea*, but the most recent, as well as the most detailed, description of them is that by HABERLANDT (22). Another paper of interest is that of DEAN (12), who, after studying *Dionaea* under native conditions, suggested that it would be more appropriate to call the plant "ant or beetle catcher" than to call it "fly-trap," because of its evident "specialization for the capture of ground insects." That the mechanism which causes the movement of the leaves has proved perplexing, is evidenced by the number of researches that have been and are still being conducted on the subject. The most recent papers offering an explanation of the movement of the leaves have been published by BROWN and SHARP (3), BROWN (4), and GUTTENBERG (21).

Dionaea can be propagated both by seed and by cuttings of the rootstock. HOLM (25) figures and briefly describes the germination of the seed. Although KURTZ states that it had long been a common practice among gardeners to propagate *Dionaea* by means of cuttings made from the inflorescence or flower stalk before the flowers

open, it was not until 1892 that this type of vegetative propagation was described and figured (HARSHBERGER **23**, **24**).

This brief summary includes the more important papers which have dealt with *Dionaea*. Several others, primarily of taxonomic interest, will be referred to later.

Habitat

Dionaea is a monotypic genus, indigenous, as reported by DARBY (10), CHAPMAN (**7**), and SMALL (**37**), to the wet sandy savannahs of eastern North Carolina and eastern South Carolina. The herbaria of the United States National Herbarium, the Missouri Botanical Garden, the New York Botanical Garden, Field Museum of Natural History, the Academy of Natural Sciences of Philadelphia, and the Gray Herbarium include specimens from the following localities in North Carolina: Wilmington, Burgaw, Lake Catherine, Bladen County, Bolton, Scott's Hill, Fayetteville, Dixon, and Hallsboro; and from Georgetown, South Carolina. Professor COKER informs me that he is publishing (**8**) a map which gives the distribution of *Dionaea*.

The writer visited eastern North and South Carolina the second week in April, 1928, for the purpose of seeing and collecting *Dionaea* in its native habitat. Plants were found growing near Wilmington in an open space in the pines, associated with *Drosera rotundifolia*, *Pinguicula lutea*, *P. elatior*, *Iris verna*, *Sarracenia purpurea*, and *S. flava*. At numerous other habitats from which *Dionaea* had been previously collected by various persons no plants were found, due no doubt to the late spring.

Material and methods

The plants of *Dionaea* used in this study were collected from one of its habitats near Wilmington, North Carolina. The material was shipped in living condition, and then either at once or after being established for some weeks in a greenhouse was fixed in a solution containing 2.5 cc. of glacial acetic acid and 6 cc. of commercial formalin to 95 cc. of 50 per cent alcohol. After dehydration it was imbedded by the usual paraffin method, cut in 5–10 μ serial sections, and stained on the slide. The stain used for the greater part of the work was Haidenhain's iron-haematoxylin. Flemming's triple stain

of safranin, orange G, gentian violet, and the double stain of safranin and light green were sometimes employed, however.

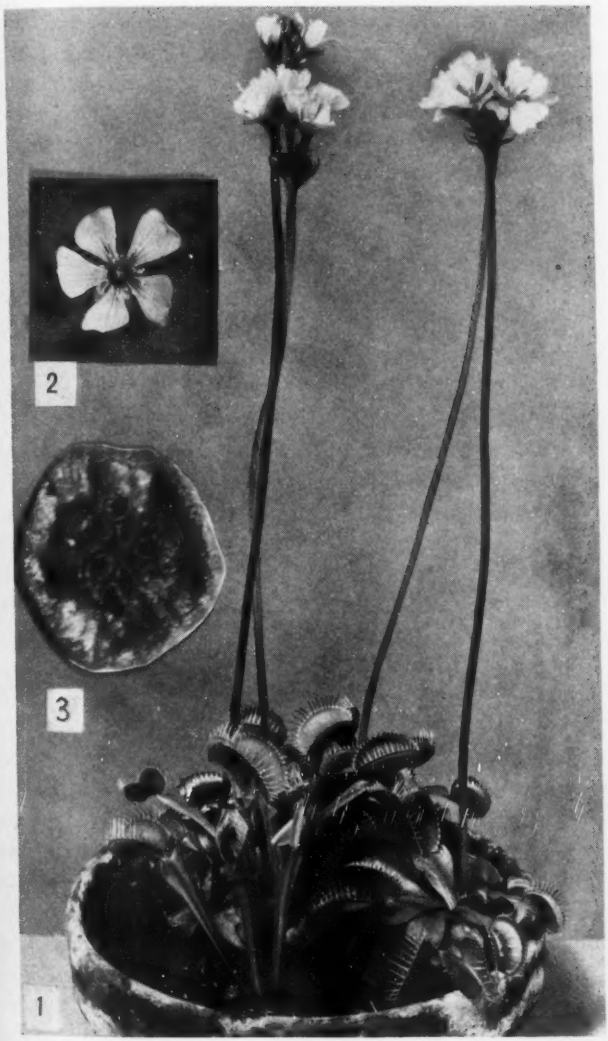
Development of inflorescence

In the early spring, the apex of the sympodial, perennial rhizome of *Dionaea* sends up successive aerial leaves. When from eight to twelve leaves have been developed, a leafless floral axis begins to push out of the terminal bud of the short, horizontal rhizome. Plants collected in North Carolina on April 15, 1927, and examined in Baltimore on April 18 showed scapes 2–3 cm. long. Plants collected April 8, 1928 in the field 2 miles from Wilmington just off the New Bern road possessed floral axes 1–2 cm. in length. When the scape is mature, it may reach 20 cm. or more in height. This axis of the mature flower stalk or scape is about 4 mm. in diameter, and bears a cyme of from two or three up to twelve or fourteen flowers (fig. 1). Not more than four or five flowers of a cyme are open at one time. The flower lasts for about three days.

Each flower has five small green, elliptical, persistent sepals pointed at the apex; five white, cuneate petals notched at the apex; eleven to fifteen stamens (usually fifteen); and a single, compound pistil composed of five carpels (fig. 2). This radially symmetrical, pentacyclic flower develops essentially in the normal manner, the five members of each perianth cycle being synchronous in origin and the different cycles appearing in the usual sequence.

Stamens and pollen grains

The third and fourth cycles of organs to be differentiated in the flower are composed of stamens. Members of the last formed, epipetalous cycle are often doubled (fig. 6*A*, *B*). Payer (33) was the first to point out that the stamens of *Dionaea* develop in two whorls. He states, however, that the alternipetalous stamens appear interior to and before the epipetalous stamens; and with regard to the number of stamens developed in each whorl he says, "Rarement chacun de ces verticilles n'est composé que de cinq etamines; le plus souvent on en compte plus de cinq, parce que à la place d'une il s'en développe deux." Moreover, in fig. 19, pl. 38, he shows a young flower in which the alternipetalous stamens are doubled, whereas in fig. 20, pl. 38, he shows a slightly older flower in which the epipetalous



FIGS. 1–3.—Fig 1, plants showing different types of leaves, mature scapes, and cymose inflorescence (note wasp captured by leaf of plant on right); reduced one-half; fig. 2, flower with 5 sepals, 5 petals, 14 stamens, and pistil composed of 5 carpels; slightly reduced; fig. 3, receptacle of mature fruit with cup-shaped bolsters of placental tissue (photographed by A. F. SKUTCH); ×15.

stamens are double. Later, in describing the stamens of *Dionaea*, EICHLER (**16**) says, "Nach PAYER gehören dieselben 2 ursprünglich 5 zähligen Kreisen an, von denen der alternipetale zuerst entsteht; bei Ueberzahl findet Dédoublement statt, *namentlich im epipetalen Quirl*[1] (fig. 88 *C*)." In this figure (p. 224) EICHLER has indicated the "dédoublement" of the epipetalous stamens which he accredits to PAYER. An examination of PAYER'S description and figures, however, makes it clear that he believed that either the alternipetalous or the epipetalous stamens could undergo "dédoublement." Although the portion of EICHLER'S statement which I have italicized agrees with the facts, it has been wrongly attributed to PAYER. DIELS (**13**), RENDLE (**34**), and others cite PAYER for this explanation, which, however, is not found in PAYER but in EICHLER. Further proof of EICHLER'S figure may be had by following from their source the course of the vascular bundles of the stamens of young flowers. If this is done, it is found that when the flower has more than ten stamens, one or more of the five vascular strands which supply the five epipetalous stamens are divided, and that two epipetalous stamens have developed in place of one where the strand is divided. Fig. 6*A* shows a cross-section of the receptacle of a young flower containing fourteen stamens, in which five vascular strands respectively of the outer alternipetalous and of the inner epipetalous stamens are visible. Fig. 6*B* shows a similar section of the same flower 60 μ nearer the apex, in which four of the five vascular strands of the epipetalous stamens are divided. If, on the other hand, the flower develops but ten stamens, the five outer alternipetalous stamens alternate with the five inner epipetalous stamens, as is shown in fig. 5*C*. Although most of the taxonomists report ten to twenty stamens for *Dionaea*, none of the flowers which I have as yet examined possessed more than fifteen. I have been unable, therefore, to determine what the arrangement of the stamens would be in a flower developing more than fifteen stamens.

Each stamen originates as a protuberance involving dermatogen and periblem. As the protuberance grows it pushes upward, and gradually broadens above the slender base (figs. 5, 7, 9). The upper enlarged end is the anther, in which four microsporangia develop; the slender base becomes the filament.

[1] Italics mine.

The microsporangia develop in the usual manner. A cross-section of a young anther shows usually a single primary archesporial cell in each of the four corners immediately beneath the epidermis. In the upper right corner of fig. 8 are shown two primary archesporial cells, a relatively rare case. A longitudinal section shows that, in reality, each cell seen in transverse section is one of a row of from five to seven cells extending the full length of the anther. Each cell of this row divides into an outer parietal and an inner sporogenous cell. By radial and periclinal divisions, the primary parietal cells then give rise to from three to five concentric layers of wall cells (fig. 10).

Subsequent differentiation among the wall cells results in the formation of the tapetum, the "middle layers," the endothecium, and the epidermis. In following the further history of each layer, changes are seen to occur first in the innermost layer of wall cells, the tapetum. Its cells enlarge, become granular, and often binucleate (figs. 16, 21). In enlarging, the tapetal cells encroach upon the "middle layer," crush, and finally absorb the one or two rows of cells of which the middle layer is composed (figs. 21, 23). Next the cells of the endothecium begin to increase in size. A transverse section of a microsporangium (fig. 23) made at this stage shows the cells of the endothecium to be approximately as large as those of the epidermis, and the tapetum greatly reduced as it is being gradually absorbed in the maturing of the spores. Finally the tapetum completely disappears, the walls of the endothecium become prominently thickened in bands, and the cells of the epidermis disintegrate (fig. 25). This same figure shows the weak place in the sporangium wall, where the slit, by means of which the pollen grains may escape, is to be formed.

By successive divisions of the primary sporogenous cells forty-eight to sixty microspore mother cells are formed. These mother cells have increased, by the time of synizesis, from a diameter of 15–17 μ to one of 25–30 μ. Concurrently the diameters of their nuclei enlarge from a diameter of 10 or 11 μ to one of 15 or 17 μ. The nucleoli do not change in size, remaining about 4 μ in cross-section. Due to the fact that the nucleus of the spore mother cell increases in size as the cell matures in preparation for meiosis, however, the

nucleolus is proportionately larger in the early stages. In a single microsporangium all nuclear changes occur almost simultaneously (fig. 16), but the four sporangia of the same stamen do not always show the same stage of development.

The nuclear phenomena which occur in the spore mother cells preparatory to and during the formation of tetrads may be described as follows. Small chromatin granules or prochromosomes, similar to those described by ROSENBERG (35) in *Drosera* and by OVERTON (31) in *Thalictrum*, *Calycanthus*, and *Richardia*, are visible in the resting nucleus. They are arranged in pairs of units lying side by side on the linin threads, and are distributed about the periphery of the nucleus, close to the nuclear wall (fig. 11). As the nucleus enters the prophase (fig. 12), the chromatin threads become more distinct and then pass into the synizesis contraction (fig. 13). The paired chromatin threads become massed together at one side of the nuclear cavity in synapsis. Usually the nucleolus lies adjacent to but distinct from the chromatin mass. In some cases, however, it is surrounded by filaments of chromatin.

When the threads of the synaptic knot loosen up, the loops of the spireme formed appear to be double, with paired chromatin granules scattered along them (fig. 14). The spireme then segments into fifteen pairs of bivalent chromosomes, which are found scattered throughout the nucleus (fig. 15). Some parts of the chromosomes stain more deeply than other parts, as is shown in fig. 15. As a consequence of gradual shortening and thickening, the chromosomes acquire a compact form and draw closer together (fig. 17). The nuclear membrane is visible about the chromosomes in diakinesis. Fig. 16 shows a longitudinal section of a microsporangium in which the cells are rounded up and more or less separated from one another (segments of the original walls being still visible between some of the cells), and in which the nuclei of all the microsporocytes are in diakinesis.

The microsporocytes of a sporangium adjacent to the one shown in fig. 16 have advanced a stage further; the spindle is formed for the first meiotic division. Fig. 18 shows the V-shaped chromosome tetrads as they appear in metaphase with the spindle fibers attached. The double character of the chromosomes, as they move apart in the anaphase, becomes clearly discernible (fig. 19). Four nuclei, each

having fifteen haploid chromosomes, result from the second division, which occurs immediately after the first (fig. 20).

Walls appear between the four nuclei (fig. 21), thus cutting out the four microspores of a tetrad. As the microspores mature each develops an outer spore coat, the exine, containing six to eight germ pores, and an inner spore coat, the intine (fig. 23). The exine is composed of an outer layer, made up of slender prismatic elements standing perpendicular to the surface, interspersed among which are longer, pointed prisms or spines; and of an inner layer of apparently homogeneous structure (fig. 26). The two layers when fully developed are of about equal thickness (each 2.5 μ). Because of the prominence of these two layers of the exine in the mature spore, they were at first mistaken for the exine and intine respectively, but on closer examination it was seen that they together make up the exine, and that the intine is a separate, much more delicate coat adjacent to and completely surrounding the protoplast. The intine is very plastic; it becomes more easily distinguishable when it grows out through the germ pores of the exine to form the pollen tube (figs. 25, 26).

In fig. 23 each microspore or very young male gametophyte of the tetrad is shown with its two spore coats incasing a resting nucleus surrounded by vacuolated cytoplasm. This nucleus divides into the generative nucleus and tube nucleus (fig. 25). This is the usual condition of the microspores when shed. In some instances the generative nucleus has already divided, however, forming the two male nuclei. Fig. 26 shows a section through a tetrad of germinating pollen grains as found on the stigma. In each grain the pollen tube protrudes from a germ pore; in two of the pollen tubes a tube nucleus is visible; the male nuclei have not as yet passed into the pollen tube. In the interior of the style can also be seen portions of pollen tubes, identified by their tubular structure and by their reaction to stain. The cell contents of the latter could not clearly be distinguished.

Carpel and seed

The last cycle of members to be formed in the flower is one of five carpels, which, like the stamens, originate as outgrowth from the periblem involving also the dermatogen. The prominence which

gives rise to the carpels is at first convex, then flat (fig. 5), and later concave (fig. 7). The concave structure is divided into five carpels, each of which in turn is differentiated into an upper and a lower region. As the upper portions elongate they curve inward, come into contact with each other, and later coalesce to form a column, known as the style, containing a central stylar canal (figs. 9, 27, 32). The end of the style is divided into numerous projections or papillae, composed of cells with large nuclei and dense cytoplasm which later serve as receptive structures for pollen.

In the young flower the papillae curve inward, forming a small knoblike structure, the stigma (fig. 52*A*). When the style has reached a length of about 2.5 mm., these papillose bifurcations of the stigma straighten up, bend outward, and expose the receptive tissue. Any pollen which at this time comes in contact with the stigma may germinate. Fig. 52*B* shows a pollen tetrad on the stigma. Later the papillae of the stigma shrivel and coil outward. They are found in this condition surmounting the dried-up style of the mature fruit.

The wall of the ovary is formed by the coalescing of the lower portions of the five carpels. The term "Parakarpe Gynäceen" has been applied by GOEBEL (**20**) to the ovary of *Dionaea*, because its carpels are joined by their margins only. As it grows, the cavity in which the anatrapous ovules are to develop gradually enlarges (figs. 9, 32, 52*A*). From the floor of the cavity of the very young ovary, numerous erect hypodermal protuberances appear in serial succession, from the inside outward, around the growing point of the flower, as illustrated and described by GOEBEL (**20**) and as shown in fig. 9. Each protuberance becomes differentiated into a basal stalklike structure, the funiculus, and terminal region, the nucellur or ovule. When the protrusion is about five cells across in transverse section and twenty-five in longitudinal section, the nucellar end becomes slightly curved; and, concurrently the first integument is initiated about its base (fig. 27). External to the first integument a second forms, while the nucellus curves progressively downward beside the funiculus. A longitudinal section of an ovule made at a slightly later stage (figs. 32, 33) shows the ring of tissue of the first integument surrounding all except the apex of the nucellus; and the

second integument, part of which is formed from the funiculus, encircling the first for about two-thirds of its length. The integuments continue to elongate and soon overtop the nucellus, the first integument drawing successively closer together over the nucellus, leaving only a narrow passage way, the micropyle.

Each integument is composed of two or three layers of cells. The layers of the inner integument, except where they overtop the nucellus, consist of thin-walled parenchyma cells which remain unspecialized. In the chazal end of the ovule, however, some of the thin-walled cells have become disorganized and replaced by air spaces (fig. 63). On the other hand, those cells which overtop the nucellus finally become heavily lignified and form an inner seed cover or lid (figs. 70, 71), which is pushed off in the course of the germination of the seed. This structure resembles that which NETOLITSKY (**29**) has described in *Drosera rotundifolia*.

Only the outermost layer of cells of the outer integument shows any specialization, the inner layers, with the exception of a group of thickened cells investing the vascular strand, remaining unmodified and difficult to distinguish from the cells of the inner integument. The former develops into an outer protective layer, made up of closely packed, radially arranged, heavy-walled, columnar cells (fig. 63). This might be called the palisade layer, because its cells resemble in form the palisade cells of a leaf; or the cells of which it is composed could be called Malphigian cells, except that the "luminous line" usually present in the latter is wanting in the seed of *Dionaea*. The external walls of the cells become progressively and evenly thickened, whereas the radial walls first become thickened in strips (fig. 63) and later in coalesced bands (fig. 71).

If the mature fruit is examined, it is seen to contain numerous seeds grouped on a basal placenta. EDWARDS (**15**) describes the seeds and their insertion as follows: "Seeds black, shining, obovate, very acute at the lower end, half buried in the cavities of the honeycombed receptacle." DRUDE (**14**) says in regard to them: "Samen zahlreich auf der gewölbten Blütenachse central eingefügt." The writer's observations confirm these descriptions, except she finds that the lower end of each seed, even when mature, is only slightly imbedded in a cup-shaped bolster of receptacular tissue. The exact

shape and arrangement of these collars of receptacular tissue are shown in the photograph of a mature floral receptacle (fig. 3). In a longitudinal section of a much younger ovary (fig. 52*A*) this tissue is seen projecting up about the micropylar end of each ovule.

The single bundle or vascular strand of each ovule develops in the funiculus, which, as has been stated, becomes fused with a part of the outer integument. The strand extends to the chalazal region of the ovule; it shows no tendency to turn or twist in its course. In the young ovule the first vascular tissue to be developed consists of narrow, elongated, densely protoplasmic cells, the procambium (fig. 33). It is from these cells that the xylem, consisting of long, slender, spirally thickened cells, and the phloem parenchyma of the mature strand develop. This mature strand, which is surrounded by a group of thickened cells of the outer integument, measures about 55 μ in transverse section.

The nucellar tissue within the integuments shows some interesting differentiations. The epidermal cells, except those immediately above the sporogenous cells, form a layer of radially placed, closely packed, thin-walled cells which enlarge immensely and in so doing become more and more conspicuous (figs. 33, 46, 58). They increase from 7.5 to 62.5 μ in anticlinal length from the time that the spore mother cell is formed (fig. 33) to the time of fertilization (fig. 58). These radially elongated cells completely surround a central strand, four to five cells wide, which extends from the prothallial cells to the vascular strand and is composed of longitudinally elongated cells (figs. 46, 58). Since the latter lie in direct contact with the vascular strand, at the chalazal end of the ovule, it seems reasonable to assume that the prothallial cells receive their nourishment through them.

This enlargement of the nucellar cells resembles that described in *Drosera rotundifolia* by Miss PACE (**32**), who states further that these cells, together with the air spaces developed in the chalazal region of the ovule, greatly reduce the specific gravity of the seed. The latter statement is hardly true with respect to the enlarged nucellar cells of *Dionaea*, because of the fate of the cells and of the spaces occupied by them. As the embryo develops in *Dionaea* the endosperm encroaches upon the nucellar tissue (fig. 58), disintegrat-

ing and absorbing both the central strand of elongated cells and the encircling radially-attenuated cells (fig. 63). Finally, in the mature seed the cellular endosperm completely fills the space occupied by the nucellus, and also the space occupied by the thin-walled cells of the integuments (fig. 71). As a result the specific gravity of the seed does not decrease but is actually increased.

Since the latest stage of seed development of *Drosera* figured by Miss PACE shows fertilization, it seems reasonable to suppose that she did not follow out the subsequent development of the endosperm and the fate of the nucellar tissue; hence her conclusion that the enlargement of the nucellar cells reduces the specific gravity of the seed is not valid. Further, an examination of a series of slides of *Drosera rotundifolia* made by C. A. PETERS at Ann Arbor, Michigan, and now in the collection of slides of the Johns Hopkins University, shows that the endosperm develops at the expense of the nucellar tissue very much as it does in *Dionaea*.

The single archesporial cell of the megasporangium is set off immediately beneath the epidermis of the nucellus, about the same time that the first integument appears. This cell is easily recognized by its large size and dense protoplasm, as well as by its apical position in the nucellus (fig. 28). It is about 17 μ in transverse section, and contains a nucleus which has a diameter of 10 μ. By mitotic division (fig. 29) it gives rise to the primary sporogenous cell and primary parietal cell (figs. 30, 31). The latter is concerned with the production of a small portion of the megasporangium wall, but not in the formation of distinct layers of wall cells as is true of the parietal cells of the microsporangium.

The first division of the primary parietal cell is by a radial wall, which is easily discerned when it is perpendicular to the plane of the section (figs. 34, 35). Although it is impossible to follow the subsequent divisions of the individual parietal cells, as they are no longer distinguishable from the adjoining nucellar cells, it is evident that by periclinial divisions two or at most three layers of cells are formed above the sporogenous cells (figs. 44, 48). These layers are at once infringed upon and absorbed by the developing megaspore (figs. 46, 51).

The primary sporogenous cell usually develops directly into a

single megaspore mother cell (figs. 33, 34); rarely there are two (fig. 35). Since no further development of the two mother cells was observed in the several hundred sections studied, it is hardly probable that these ever progress far enough to develop two embryo sacs or embryos in an ovule. The single mother cell, on the other hand, increases in size and passes through nuclear changes similar to those described in the microspore mother cells. Paired prochromosomes, of approximately the same number as the definitive chromosomes, are visible in the resting nucleus. In the late prophase the chromatin threads become more distinct and then pass into the synaptic contraction. Fig. 36 shows the loosening up of the spireme and the paired chromatin centers about which the chromosomes develop. The spireme is then segmented into fifteen pairs of bivalent chromosomes, each of which becomes more distinct as diakinesis approaches (fig. 37). The formation of the spindle and further nuclear phenomena in the division of the megaspore mother cell were not observed. In fig. 47, however, it is evident that the reduced number of chromosomes is present at the second division of the functional megaspore; in other words, there are fifteen haploid chromosomes.

The mother cell divides to form two (fig. 38), then by a second division of one of the daughter cells it forms three (fig. 39); or in other cases both daughter cells divide and four potential megaspores are formed (figs. 41, 42). These four spores are usually arranged in an axial row. That the two daughter cells do not necessarily divide simultaneously to form the tetrad of megaspores is shown by figs. 39 and 40. Three of the four potential megaspores degenerate, leaving but one functional. Usually in *Dionaea*, as in other angiosperms, the non-functional cells of the micropylar end of the ovule disappear (figs. 44, 48, 49). Fig. 43 shows a single active megaspore with the degenerating cells in the chalazal end of the ovule, an uncommon occurrence. The last vestige of the degenerating megaspores disappears before the eight-nucleate embryo sac is completely organized.

Embryo sac, endosperm, and embryo

The embryo sac is initiated with the division of the functional megaspore, after which the daughter nuclei move apart, one going to each end of the embryo sac. The cytoplasm between the nuclei

becomes more and more vacuolated (figs. 44, 45). Subsequent divisions take place rapidly and simultaneously. Fig. 48 shows the four nuclei and fig. 50 the eight nuclei resulting respectively from the second and third divisions; and fig. 49 shows that the third division occurs at the same time in all four of the nuclei resulting from the second one.

One of the four nuclei at each end of the embryo sac, the polar nucleus, migrates toward the center. The three nuclei remaining at the micropylar end form the egg apparatus; the three nuclei remaining in the chalazal end form the antipodal cells (fig. 51). The egg apparatus is quite normal, consisting of two synergids and an egg. Each of the former has a notch, is capped by a filiform apparatus, and contains one or more vacuoles in its lower end (fig. 51). The egg cell has a larger nucleus than the synergids, and usually lies nearer the center of the embryo sac, below the synergids. The two polar nuclei, which come to rest in the middle of the sac just below the egg, are of about the same size, each containing a large nucleolus. The two nuclei may lie side by side at the same level, or one may be nearer the egg and the other nearer the antipodals. They are always surrounded by a mass of cytoplasm, some strands of which extend upward to connect with the layers of cytoplasm that surround the lower end of the egg, while other strands reach to the notches in the synergids. The antipodal cells which are located in the chalazal end of the sac never become conspicuous; they often disappear shortly after they are formed.

Actual fusion of the egg and sperm cells was not observed, although hundreds of sections, made at about the time of fertilization, were examined. Fig. 51 shows the polar nuclei in contact with each other, and fig. 53 shows the polar nuclei in the process of fusion, thus forming the primary endosperm nucleus. In some cases the polar nuclei fuse before, and in other instances after fertilization. Fig. 54 shows the primary endosperm nucleus with its characteristically large nucleolus. The mean diameters of the primary endosperm nucleus and nucleolus are 18 and 8 μ respectively, ascertained by averaging twenty measurements. Referring again to fig. 54, the fertilized egg can be seen below the synergids, the contents of which are entirely obscured by a darkly staining substance, that was probably

introduced with the entrance of the pollen tube. That the synergids do not disappear immediately after fertilization is evidenced by the fact that remains of them and their degenerating nuclei are visible after several divisions of the fertilized egg (figs. 59, 61).

The primary endosperm nucleus divides before the fertilized egg, and its subsequent divisions occur more rapidly than those of the egg. The nuclei resulting from the first and second divisions of the endosperm nucleus are shown in figs. 55 and 57; they are elongated and each contains two or more nucleoli. The egg has not yet divided. The early mitoses occur simultaneously in all nuclei (fig. 56). Later, however, when the endosperm has become multinucleate, the nuclei in one region show a different stage of development from those in other regions of the endosperm (fig. 63).

With the multiplication of the nuclei in the endosperm, the cytoplasm surrounding the former becomes more and more vacuolated; and, as a result of the union of numerous small vacuoles, a single sap-filled central vacuole develops. With this increasing vacuolation the nuclei are caused to assume a peripheral position, a group of them becoming arranged in a funnel-shaped mass near the chalazal end of the embryo sac. From fig. 58 it will be seen that, although the fertilized egg has not divided nor have the synergids completely disintegrated, the endosperm has progressed to the condition just described, the lower funnel-shaped portion of it having already begun its inroads upon the surrounding nucellar tissue. As growth continues this lower end of the endosperm, which acts somewhat as a haustorium, progressively encroaches upon and absorbs the nucellar cells, until it finally comes in direct contact with the vascular strand at the chalaza. A median longitudinal section through the ovule (fig. 63) shows a relatively thin layer of cytoplasm, with free nuclei surrounding a central vacuole and extending from the embryo to the vascular strand. A mass of cytoplasm and nuclei are to be seen in contact with this strand. A surface view of the endosperm, made at the same stage (fig. 64), shows the nuclei about equally spaced in a uniformly distributed layer of cytoplasm.

When free nuclear division has ceased in the endosperm, walls are formed between the nuclei, as a result of which an outer layer of cells is developed about the central cavity (fig. 69). With the

initiation of wall formation, each nucleus of the hundred or more then present becomes surrounded by a somewhat denser mass of cytoplasm, strands of which extend to the encircling cytoplasm of the adjacent nuclei (figs. 65, 66). Each of the connecting strands becomes less dense and more curved as it passes outward from the mid-line between adjoining nuclei. STRASBURGER (38) has described and figured, in *Agrimonia eupatoria*, *Reseda odorata*, and certain other plants, a condition similar to this. The distribution of the cytoplasm about the nuclei at this stage makes it possible to predict that the cell walls will appear in a plane equidistant between neighboring nuclei and at right angles to the connecting strands. The next stage observed is shown in fig. 67. The fibrillae, which had their origin in the connecting strands of cytoplasm, show the equatorial thickenings which later split to form two parallel cell plates (fig. 68). A thin continuous cell wall is soon formed between the two cell plates and the fibrillae begin to disorganize. The cells thus formed now divide and gradually replace the central cavity (fig. 69). Finally the entire embryo sac, except the part occupied by the embryo, is filled with the cellular endosperm (figs. 70, 71).

In contrast to the free nuclear division of the primary endosperm nucleus, the first division of the nucleus of the fertilized egg is accompanied by the formation of a cell wall. This first division is transverse to the long axis of the embryo sac, moreover, and results in the formation of the suspensor and embryo (fig. 59). Of the two the suspensor cell is situated nearer the micropyle, is larger, and tapers toward the micropyle, while the embryo proper is hemispherical. Furthermore, the first division of the suspensor cell usually precedes that of the egg (fig. 60).

The first and second divisions of the embryo are longitudinal and transverse respectively. Fig. 61 shows the four cells resulting from these divisions. The cytoplasm of the cells of the embryo is much denser than that of the suspensor cells. The cells of the embryo continue to multiply, and soon an outer layer of cells, the dermatogen, is cut off by periclinial walls (fig. 70). In the meantime the suspensor cells have divided and formed a cylindrical structure composed of six to eight cells (fig. 70), the ultimate fate of which was not determined. The embryo in the mature seed consists solely of two small

cotyledons and a short radicle (fig. 71). The structure of the embryo becomes further differentiated in the process of germination. It will be more fully described in a second paper, on *Dionaea muscipula*, dealing specifically with the germination of the seed and seedling. The bearing of the facts here recorded on the relationships of *Dionaea* will also be deferred to the second paper for discussion.

Summary

1. The terminal bud of the short perennial rhizone of *Dionaea* forms a scape which bears a cymose inflorescence of from two to fourteen actinomorphic, pentacyclic flowers.

2. The arrangement of the vascular bundles of the stamens in the receptacle of young flowers is considered as further proof of PAYER'S statement that the stamens develop respectively in alternipetalous and epipetalous whorls. Moreover, when more than ten stamens (five in each cycle) develop, the vascular bundle of one or more of the five epipetalous stamens is divided and supplies the additional stamen or stamens. This is not in accord with the view held by PAYER that "dédoublement" may occur in either whorl.

3. The four microsporangia of each stamen develop in the usual manner.

4. In the early prophase of the microsporocytes, of which forty-eight to sixty develop in each sporangium, prochromosomes are discernible.

5. Each of the four nuclei resulting from the second meiotic division of the microsporocyte has fifteen haploid chromosomes.

6. The mature pollen grains hang together in tetrads and each contains six to eight large germinating pores.

7. The "paracarpous" ovary of *Dionaea*, which is composed of five carpels, develops numerous anatropous ovules on a basal placenta, with the tip of each slightly imbedded in a cup-shaped bolster of placental or receptacular tissue.

8. The outermost layer of cells of the second integument forms an outer seed coat of closely packed, radially elongated, heavy-walled, columnar cells; while those cells of the inner integument which overtop the nucellus form a lid or cover which is pushed off when the seed germinates.

9. The single vascular strand which extends to the chalazal end of each ovule shows no tendency to turn or twist in its course.

10. The outer layer of nucellar cells, except where it overtops the prothallial cells, increases enormously in radial dimension, surrounding a central strand of longitudinally elongated cells.

11. By division of the single hypodermal archesporial cell, two or at most three layers of parietal cells are formed above the embryo sac.

12. Rarely more than a single megaspore mother cell is developed; the mother cell divides to form two, then four potential megaspores; the divisions of the daughter cells do not necessarily occur simultaneously.

13. Usually, as in other angiosperms, the three micropylar potential megaspores degenerate and the chalazal one functions. In one instance the three basal spores degenerated and the micropylar one functioned.

14. The embryo sac resulting from three successive divisions of the functional megaspore contains, when ready for fertilization, the usual egg apparatus, polar nuclei, and antipodals.

15. The primary endosperm nucleus usually completes several mitoses before the fertilized egg divides to form the embryo and suspensor.

16. As the endosperm increases it progressively encroaches upon the surrounding nucellar tissue, and by the time that free nuclear division has ceased in the former all nucellar tissue has disappeared.

17. The mature seed contains a small basal embryo, consisting of two cotyledons and a short radicle surrounded by the cellular endosperm.

This study was undertaken at the suggestion of Professor DUNCAN S. JOHNSON, whose generous interest and invaluable direction have been indispensable to its prosecution. My indebtedness to him I cannot too gratefully acknowledge

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EXPLANATION OF PLATES XX–XXIV

All figures were drawn with the aid of the camera lucida, except figs. 1–3.

FIG. 4.—Transverse section of very young inflorescence; sepals differentiated in all flowers, petals in lowest flower on left only; ×38.

FIG. 5.—Longitudinal section of young flower, showing protuberances giving rise to stamens and carpels; ×38.

FIG. 6.—*A*, transverse section of receptacle of young flower containing 14 stamens, showing the 5 vascular strands respectively of outer alternipetalous and inner epipetalous stamens; *B*, similar section of same flower 60 μ nearer apex, in which 4 of the 5 vascular strands of epipetalous stamens are divided; *C*, similar section of flower developing only 10 stamens, 5 outer alternipetalous

alternating with the 5 inner epipetalous (*s*, sepal; *p*, petal; *a*, alternipetalous; *e*, epipetalous); all ×18.

FIG. 7.—Longitudinal section of flower of about same age as those shown in preceding figure; stamens clavate, carpels concave; ×38.

FIG. 8.—Transverse section of young anther, showing 2 primary archesporial cells in left corner, primary archesporial cell in mitosis in right corner, and primary parietal and sporogenous cells in each of lower corners; ×172.

FIG. 9.—Longitudinal section of flower with stamens in spore mother cell stage and ovules appearing in base of ovary; ×38.

FIG. 10.—Transverse section of single anther; microspore mother cells surrounded by 3 or 4 concentric layers of wall cells; ×172.

FIG. 11.—Single microspore mother cell in early prophase, showing paired prochromosomes and nucleolus; ×925.

FIG. 12.—Nucleus in late prophase; ×925.

FIG. 13.—Nucleus in synapsis; ×925.

FIG. 14.—Nucleus with uniformly distributed, distinctly double spireme; ×925.

FIG. 15.—Spireme segmented; 7 pairs of chromosomes shown; ×925.

FIG. 16.—Longitudinal section of microsporangium, showing binucleate, granular tapetal cells and rounded-off microsporocytes in diakinesis; ×172.

FIG. 17.—Nucleus of microsporocyte, showing chromosomes in diakinesis; ×925.

FIG. 18.—Chromosomes in late metaphase of meiosis number one; ×925.

FIG. 19.—Polar view of anaphase of first meiosis; double nature of chromosomes clearly visible; ×925.

FIG. 20.—Four nuclei, each with 15 haploid chromosomes, resulting from second meiotic mitosis; ×925.

FIG. 21.—Transverse section of microsporangium, showing epidermis, endothecium, "middle layers," and tapetum consecutively surrounding tetrads of pollen grains; ×172.

FIG. 22.—Transverse section of anther, showing relative position of 4 microsporangia; ×18.

FIG. 23.—Detail of single microsporangium: epidermis, endothecium, disintegrating tapetum, and tetrads of pollen grains with germinating pores, nucleus, and vacuolated cytoplasm distinguishable; ×172.

FIG. 24.—Transverse section of almost mature anther, showing position of the 4 microsporangia, and partition wall between adjacent sporangia; ×18.

FIG. 25.—Detail of single microsporangium, showing weak spot where split will occur, endothecium with its thickened fibers, and mature pollen grains; ×172.

FIG. 26.—Tetrad of germinating pollen grains; ×715.

FIG. 27.—Median longitudinal section of young pistil; ovules on basal placenta, surrounding growing point of flower; ×38.

FIG. 28.—Single hypodermal archesporial cell of megasporangium; ×715.

FIG. 29.—Archesporial cell in mitosis; ×715.

FIG. 30.—Primary parietal and sporogenous cells; ×715.

FIG. 31.—Similar section but of somewhat differently shaped cells; ×715.

FIG. 32.—Longitudinal but not quite median section of slightly older pistil; ×38.

FIG. 33.—Detail of single ovule, showing procambium, outer and inner integuments, and megaspore mother cell; ×172.

FIG. 34.—Megaspore mother cell in early prophase, with two parietal cells immediately above; ×715.

FIG. 35.—Similar section, showing two megaspore mother cells; ×715.

FIG. 36.—Nucleus of megaspore mother cell, just previous to segmentation of spireme; ×925.

FIG. 37.—Chromosomes in diakinesis, 15 pairs visible; ×925.

FIG. 38.—Two megaspores resulting from first division of mother cell; ×715.

FIG. 39.—Similar stage; nucleus of one cell in mitosis; ×715.

FIG. 40.—Similar stage; nucleus of each cell in different stage of mitosis; ×715.

FIG. 41.—Similar stage; nucleus of each cell in same stage of mitosis; ×715.

FIG. 42.—Similar stage; 4 nuclei in early prophase; no walls formed between grand-daughter nuclei; ×715.

FIG. 43.—Functional megaspore in micropylar end of ovule; 3 degenerating megaspores in chalazal end (only 2 shown in this figure, third in next section); ×715.

FIG. 44.—Longitudinal section of 2-nucleate embryo sac; nuclei migrated to each end; cytoplasm containing numerous vacuoles; ×715.

FIG. 45.—Slightly older embryo sac; nuclei in diakinesis; one large vacuole in center; ×715.

FIG. 46.—Longitudinal section of ovule containing 4-nucleate embryo sac; inner integument overtops nucellus, leaving only narrow passage way (micropyle); outer layer of nucellar cells begun to elongate radially about central core of longitudinally attenuated cells; ×172.

FIG. 47.—Detail of single nucleus, showing 15 haploid chromosomes; ×925.

FIG. 48.—Slightly different stage but similar section of 4-nucleate embryo sac; non-functional megaspores in micropylar end of sac; ×715.

FIG. 49.—Similar section, showing 4 nuclei in mitosis; disintegrating megaspores in micropylar end; ×715.

FIG. 50.—Eight-nucleate embryo sac; ×715.

FIG. 51.—Mature embryo sac containing synergids, egg, polar nuclei, and antipodals; ×715.

FIG. 52.—*A*, longitudinal, median section of pistil (just previous to fertilization), showing incurved papillae of stigma, stylar canal, and ovules with tips

slightly imbedded in cup-shaped bolsters of placental tissue; *B*, similar section of stigma with two tetrads of pollen grains attached and germinating; ×18.

FIG. 53.—Longitudinal section of embryo sac, showing fertilized egg, and two polar nuclei in process of fusion; ×715.

FIG. 54.—Slightly older embryo sac, showing fertilized egg, synergids containing dark-staining substance, and primary endosperm nucleus; ×715.

FIG. 55.—Fertilized egg and 2 nuclei resulting from first division of primary endosperm nucleus; ×715.

FIG. 56.—Endosperm nuclei in telophase of second mitosis; ×715.

FIG. 57.—Fertilized egg showing 3 of the 4 nuclei resulting from second division of endosperm nucleus; ×715.

FIG. 58.—Longitudinal section of entire ovule, showing radially elongated nucellar cells surrounding central strand of longitudinally attenuated cells, fertilized egg, synergids, and endosperm which is making inroads upon nucellar tissue; ×172.

FIG. 59.—Longitudinal section, showing suspensor cell and hemispherical embryo; ×715.

FIG. 60.—Similar section, mitosis occurring in suspensor cell; ×715.

FIG. 61.—Four-celled embryo; ×715.

FIG. 62.—Somewhat older embryo; dermatogen in process of differentiation; ×715.

FIG. 63.—Longitudinal section of entire ovule, showing 16-celled embryo, outer seed coat with thickened walls, air spaces, and multinucleate endosperm; ×172.

FIG. 64.—Surface section of endosperm shown in preceding figure; nuclei in mitosis surrounded by evenly distributed cytoplasm; ×715.

FIG. 65.—Each nucleus surrounded by denser mass of cytoplasm, strands of which extend to each of neighboring nuclei; ×715.

FIG. 66.—Slightly advanced condition, showing fibrillae between nuclei; ×715.

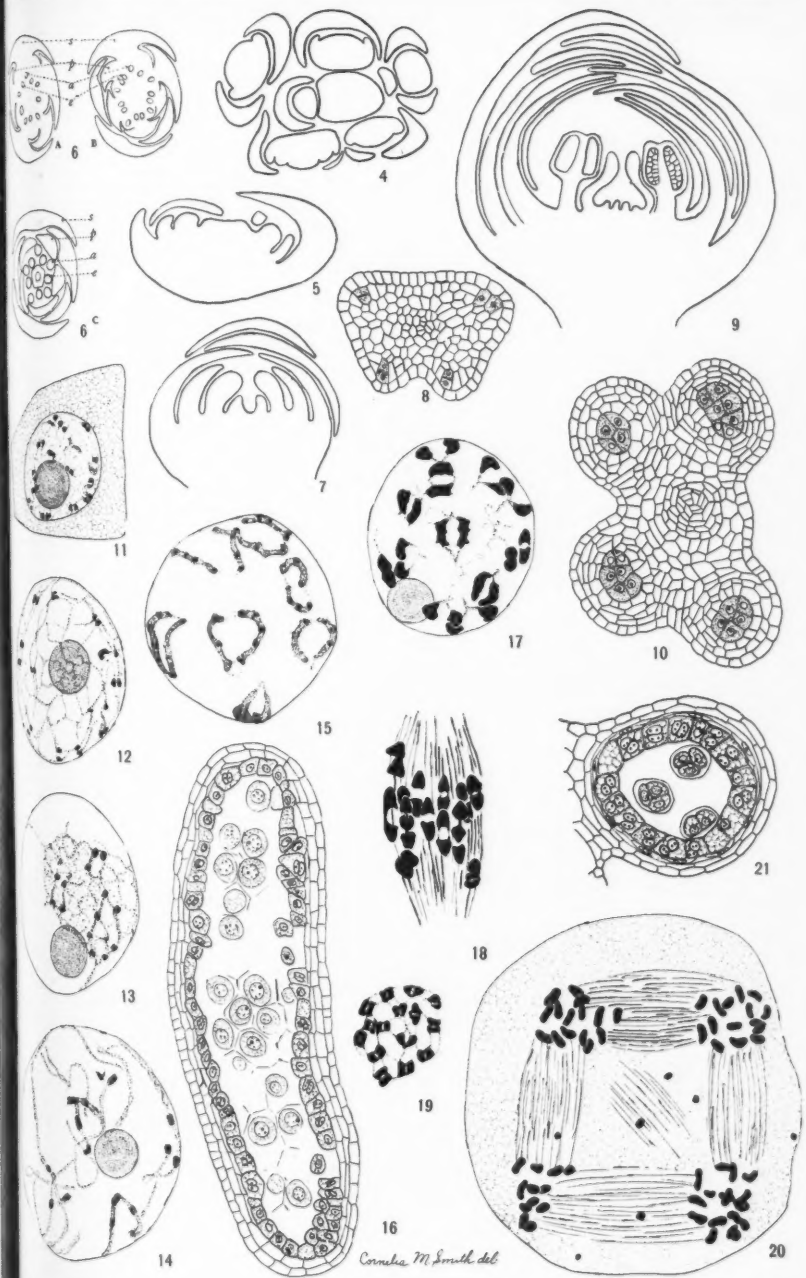
FIG. 67.—Equatorial thickenings on fibrillae; ×715.

FIG. 68.—Parallel cell plates formed by splitting of equatorial thickenings; ×715.

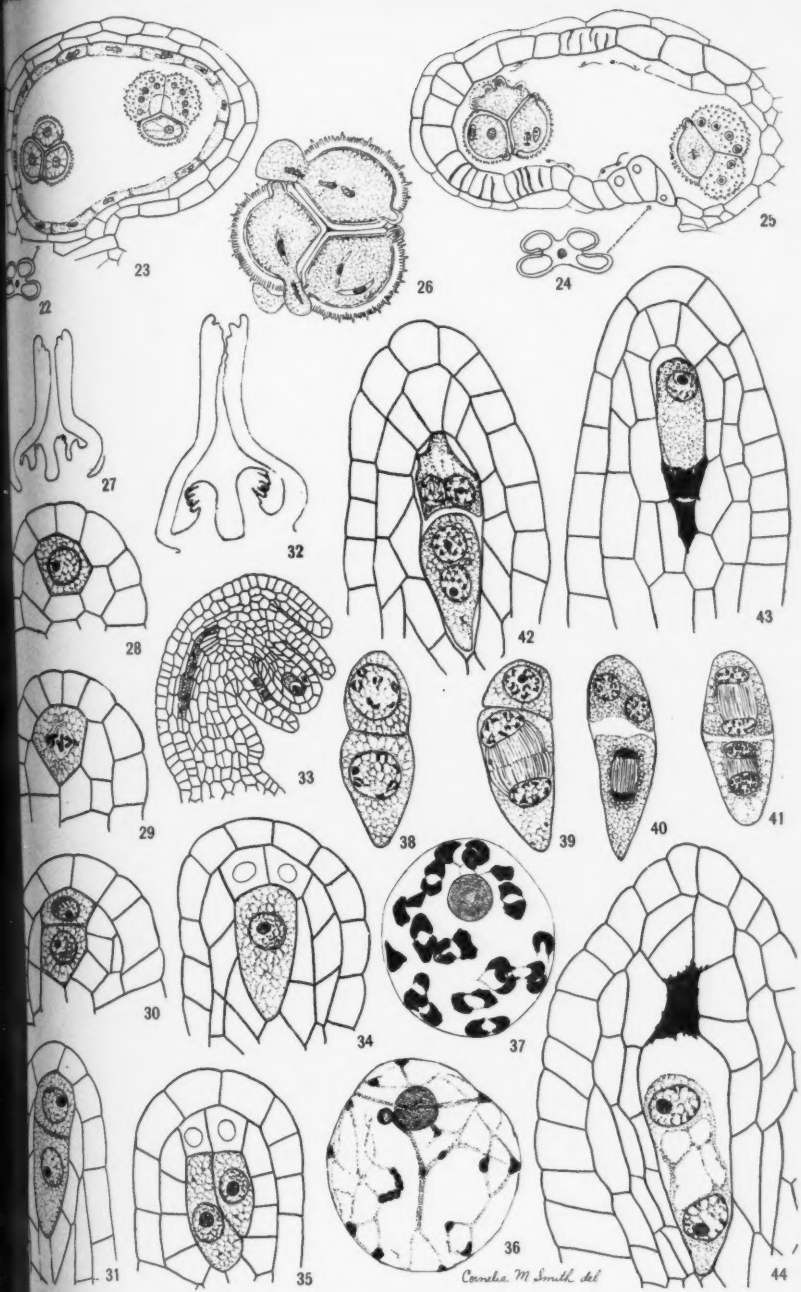
FIG. 69.—Longitudinal section of embryo and endosperm consisting of layer of cells surrounding central sap-filled cavity; ×172.

FIG. 70.—Similar section of somewhat later stage, showing seed lid formed by tip of inner integument, and cellular endosperm which completely fills central cavity and surrounds embryo; ×172.

FIG. 71.—Similar section of mature seed, showing embryo consisting of 2 small cotyledons and short radicle surrounded by single layer of cellular endosperm which fills remainder of seed; and thickened bands of cells of outer integument which have coalesced, forming black, shiny seed coat; ×73.



SMITH on DIONAEA



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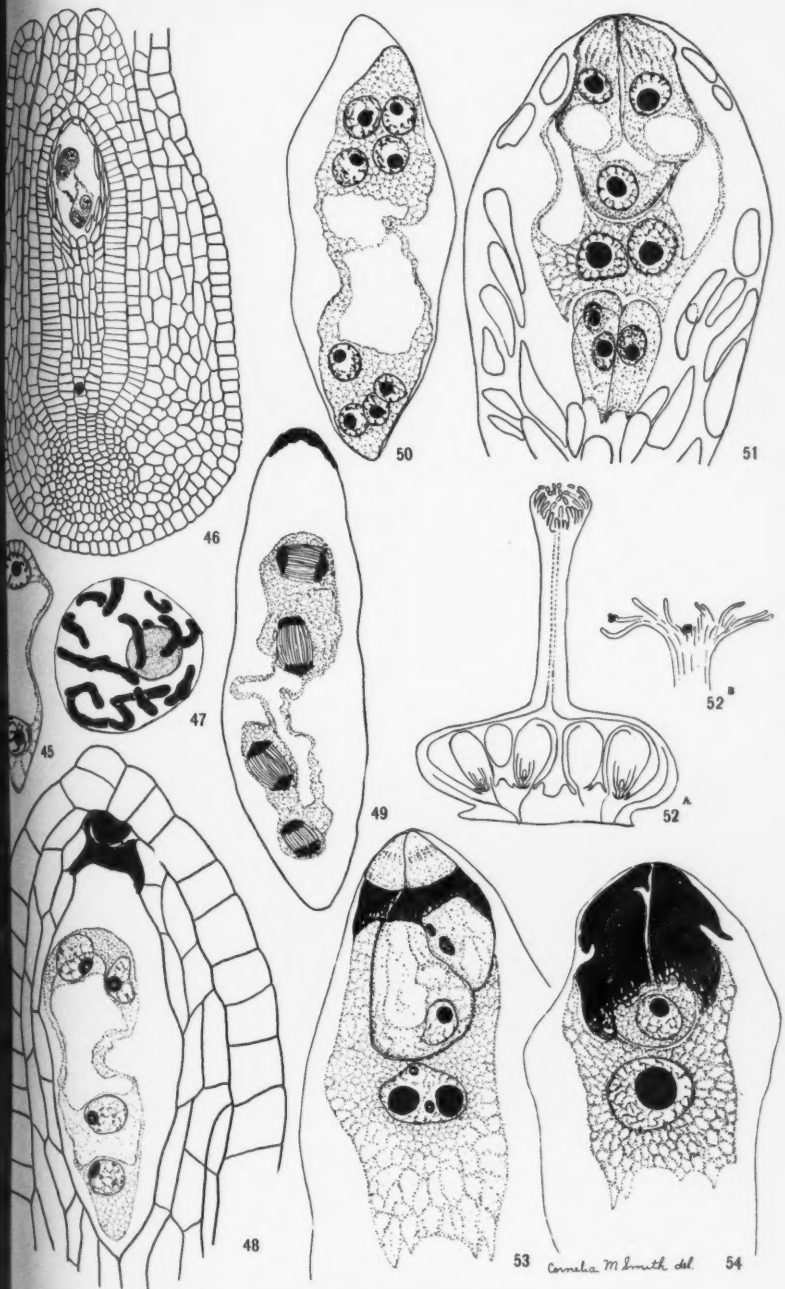

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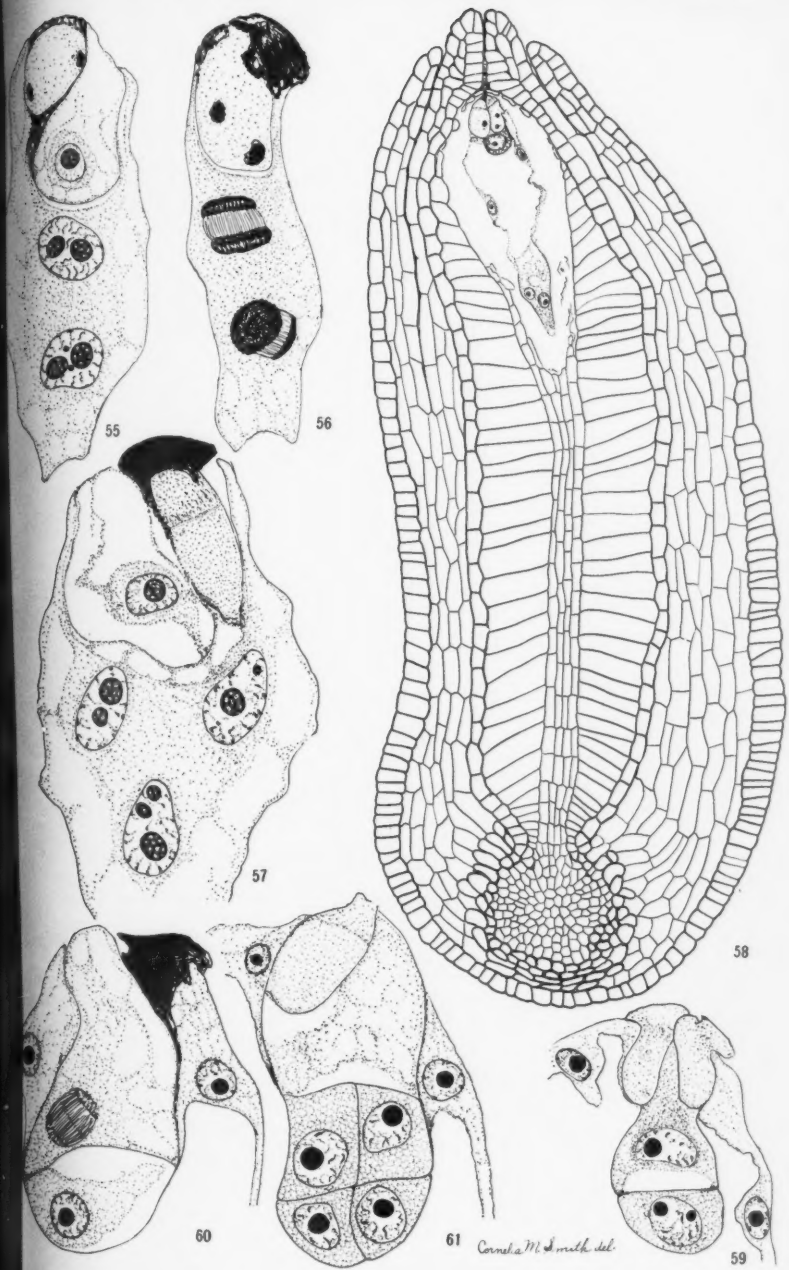

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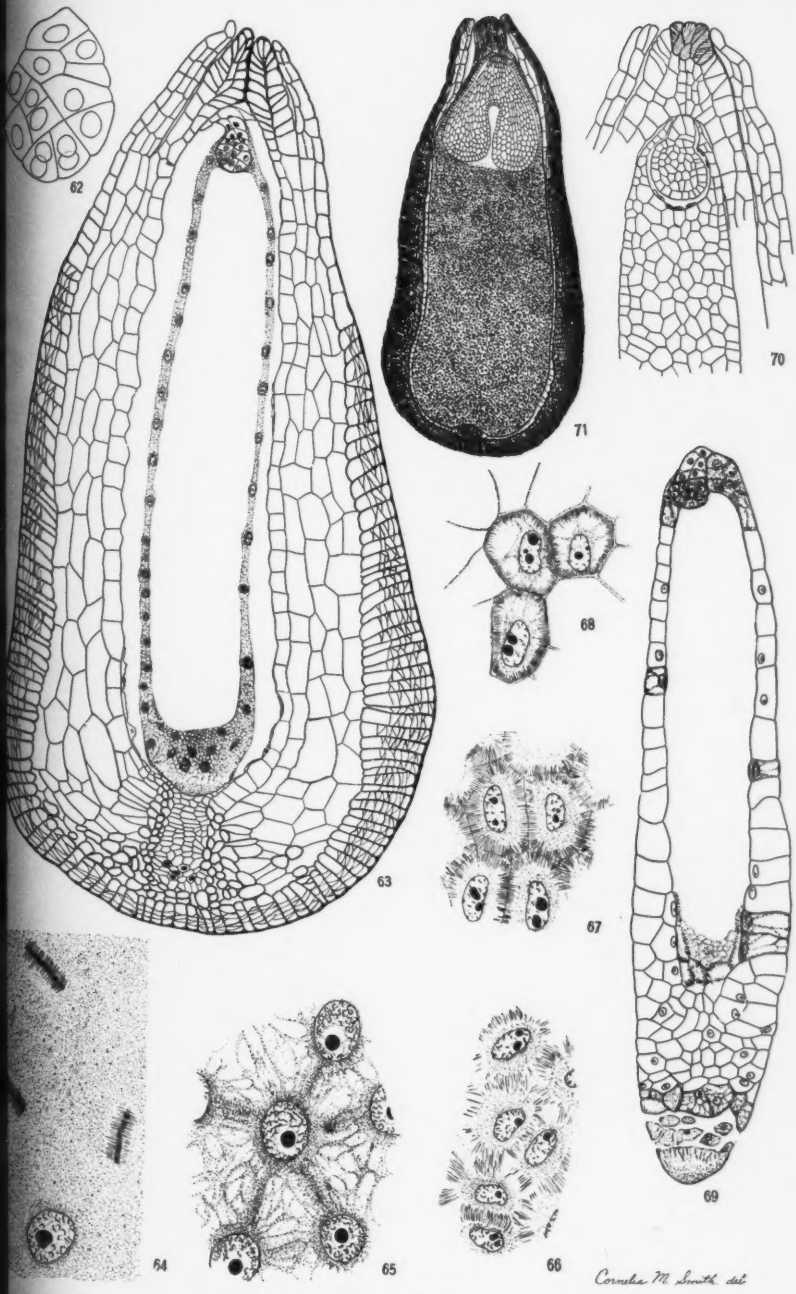

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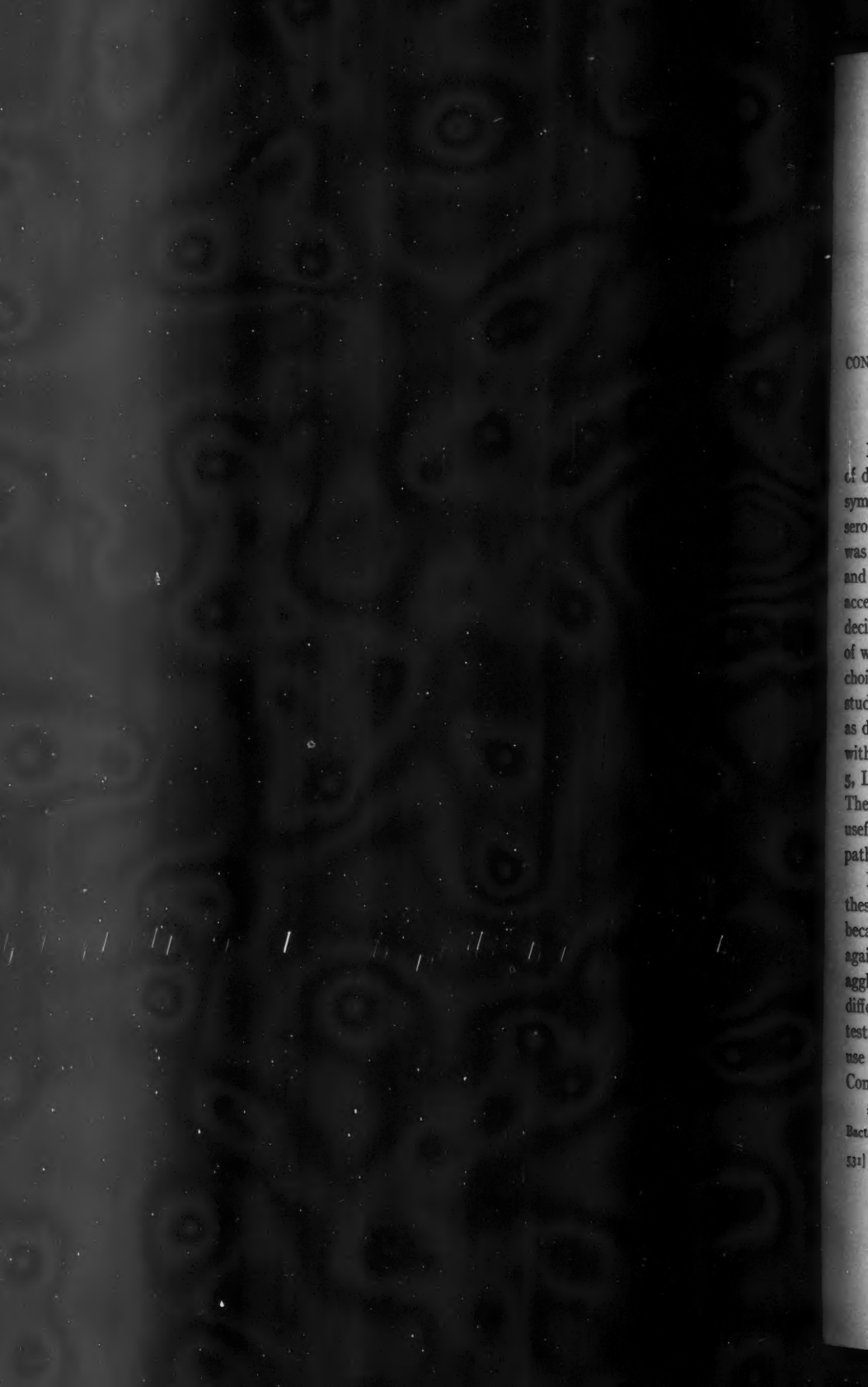

FURTHER AGGLUTINATION TESTS WITH PHYTOPATHOGENIC BACTERIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 390

G. K. K. LINK, A. E. EDGECOMBE, AND J. GODKIN[1]

Introduction

In the course of investigations in this laboratory on the nature of disease resistance in plants, the question arose whether host or symptom specificity of bacterial plant pathogens is correlated with serological specificity. To put the question to experimental test, it was deemed desirable to work with bacteria which morphologically and culturally are practically identical, that is, which according to accepted bacteriological criteria are closely related, but which show decided host or symptom specificity. The yellow pigmented group of which *Bact. campestre* is considered a member was selected. The choice proved fortunate, for it was found that for the organisms studied, with the exception of one (see later), serological specificity as determined by the direct agglutination test is indeed correlated with host specificity (LINK and SHARP **6**, SHARP **8**, LINK and LINK **5**, LINK and TALIAFERRO **7**), and even with symptom specificity. The results also indicate that the agglutination test gives promise of usefulness in the identification and classification of bacterial plant pathogens.

It was the purpose of this investigation to continue and extend these exploratory studies. This seemed desirable, in the first place, because in one instance (tests with antisera of *Bact. phaseoli sojense* against suspensions of *Bact. malvacearum*) it was found that cross agglutination was so high that the direct agglutination test was not differentiating. Furthermore, in the previous experiments reciprocal tests were not run with some of the organisms, that is, we did not use antisera of *Bact. cucurbitae*, *Bact. citri*, *Bact. pruni*, and *Bact. X*. Completely reciprocal tests have now been made with all of these

[1] We are indebted to Dr. W. H. TALIAFERRO of the Department of Hygiene and Bacteriology for cooperation in providing space for our experiments and animals.

excepting *Bact. citri*. Another gap in our exploratory studies was due to the fact that none of the cereal pathogens which are supposed to be a part of this group had been included in the tests. One of us (Mr. GODKIN) was making a comparative study of the pathogenicity as well as of the morphological and cultural characteristics of two of these cereal pathogens, *Bact. translucens* and *Bact. translucens* var. *undulosum*, which are reported to differ primarily in their host relations. It was decided not only to determine serologically whether these organisms could be differentiated, but also to determine their relationship to other members of the yellow group. To put the method to a more severe test, we included another yellow organism obtained from Dr. C. S. REDDY, the identity of which was unknown to us, but which was isolated from cereals. This project was undertaken with Mr. GODKIN.

The most intriguing reason for additional studies arose out of our earlier fragmentary study of *Bact. X*. This organism was sent us, after we had reported on our first studies (**6**), by Dr. E. F. SMITH, with the suggestion that it be included in our experiments. It arrived too late for complete reciprocal tests but was tested against the antisera of various yellow organisms (**5**). The senior author wrote to Dr. SMITH (March 14, 1927) regarding the relationship of this organism as follows: "Very clearly it is serologically closely related to *Bact. campestre*. It is also closely related to *Bact. phaseoli*, but much less closely." Later, when the paper reporting these results was in preparation, inquiry was made in SMITH's laboratory as to whether the organism was considered a *Bacterium* or a *Bacillus*, and whether it had been named. The following reply was received from Miss LUCIA MCCULLOCH: "It was isolated from a leaf spot disease of horse radish and . . . . culturally and morphologically it is very close to or identical with *Bact. campestre*."[2] Later Miss MCCULLOCH sent us a culture of another undescribed yellow organism, isolated from poppy by Miss M. K. BRYAN, with the note: "All these organisms, from squash, poppy and horse radish, are very much like *Bacterium campestre* in morphological and cultural char-

[2] This organism, here designated *Bact. X*, has now (at the time of reading proof) been described as *Bact. campestre* var. *armoraciae* n. var. McCulloch. MCCULLOCH, LUCIA, Jour. Agric. Res. **38**: 269–287. 1929.

acters. We find some slight differences and if you can find other differences in serological tests, it will help us very much in differentiating the organisms." Consequently, we decided to use the direct agglutination test and determine whether it would differentiate between *Bact. X* (horse radish), *Bact. Y* (poppy), *Bact. cucurbitae* (squash), and *Bact. campestre* (cabbage); and also to determine what the test would reveal regarding their relationships to other members of the so-called *Bact. campestre* group. In addition to *Bact. campestre*, *Bact. phaseoli* and *Bact. pruni* were selected as reference points of relationship, because BROOKS, NAIN, and RHODES (3) had reported that *Bact. campestre* and *Bact. phaseoli* are members of one group, but that *Bact. pruni* is not related to either of these or to any of the yellow organisms they studied. This phase of the work was undertaken with Mr. A. E. EDGECOMBE.

To make the results of these two projects more significant, the investigations were combined. Assistance was given by Dr. ADELINE DESALE LINK, so that we were able to complete the tests of normal sera in one day's run, and the tests with the antisera in another, each of which involved setting up almost 2000 tubes with saline, serum, and bacterial suspensions, and all the necessary preliminary dilutions of sera. By careful organization and division of labor we were able to make about 2000 tests in nine or ten hours of uninterrupted work. The advantage of this method was that all tests were run with the same suspensions, sera or antisera, so that differences due to age of sera, cultures, and density of antigen suspensions were eliminated.

The cultures of *Bact. campestre*, *Bact. phaseoli*, *Bact cucurbitae*, *Bact. pruni*, and *Bact. X* were of the stock used previously (5). *Bact. Y* was obtained from Miss LUCIA MCCULLOCH with the note that it had been isolated from poppy by Miss M. K. BRYAN. *Bact. translucens* and *Bact. translucens* var. *undulosum* were of the stock used by Mr. GODKIN in his experiments, and *Bact. Z* was obtained from Dr. C. S. REDDY.

Agglutination tests

The experimental procedure was the same as that reported in earlier papers (5, 7), except for the differences noted here. It was the intention to use antisera produced by six intravenous injections,

but this could not be done consistently because a considerable number of animals were lost at the very beginning of the experiments. Whether these losses were in part due to reaction of the animals to the antigens is not known because some of the uninjected animals also died. We are of the opinion that most of the deaths were due either to an infection which swept through the entire stock or to the extreme heat in the animal room (it was in July and August), or to both. As a result of the loss of some of the injected animals shortly after the first injection, we were confronted with the alternatives of preparing antisera in exactly the same manner and then using antisera of different ages in tests run at one time, of running the tests at different times, or of making the tests at one time but of speeding up the injections of the new animals. The last course was decided upon, and consequently some of the animals were injected intraperitoneally five times with 1 cc. of suspension, the first dose thus being double that of the first intravenous injection, and the others the same as the last five intravenous injections. The animals injected intraperitoneally are starred in the protocol. For all injections, as well as for the suspensions used in the tests, suspensions were prepared to have the turbidity of a standard nephelometer tube, which is supposed to contain 1,800,000 units per cc. As control in all series, a sample of serum drawn before immunization of each animal was tested against suspensions of the organisms to be used in tests against the antisera obtained from that animal. In making the tests, the following final dilutions of antisera were used: 1–80, 1–160, 1–320, 1–640, 1–1280, 1–2560, 1–5120, 1–7680, 1–10,240, 1–15,360, and 1–20,480. The readings were made macroscopically and with a hand lens after incubating the mixtures at 37° C. for one hour in a serological bath, and also after keeping them one hour, two hours, and twelve hours in a refrigerator. The last of these readings are recorded in table I. The protocols of the tests of the normal sera and of the antisera are not given in detail as in previous papers; instead only the titres are indicated, these being taken as the highest dilution of serum or antiserum in which any agglutination occurred as read with the unaided eye and the hand lens. The normal sera which gave agglutination are indicated in table I.

TABLE I

TITRES OF AGGLUTINATION TESTS

ANTISERUM

SUSPENSION OF ORGANISM (ANTIGEN)	BACT. CAMPESTRE		BACT. X		BACT. PHASEOLI		BACT. CUCURBITAE		BACT. Y		BACT. PRUNI		BACT. TRANSLUCENS		BACT. TRANSLUCENS VAR. UNDULOSUM		BACT. Z	SALINE CONTROL
	32	28	29	42	34	33a*	30	39	47	50	4*	450*	40	41	44	45	48	
Bact. campestre	1–768§	1–10240	1–5120	1–2560	0	0	0	0	1–1280	1–2560	0	0	0	0	1–160	1–160	0	0
Bact. X	1–5120	1–768§	1–10240	1–5120	1–320	1–80	0	0	0	1–640†	0	0	0	0	1–160†	1–160	0	0
Bact. phaseoli	1–640	1–320	0	1–160	1–320	1–320	0	1–80	0	0†	0	1–80	0	0	1–1280	1–640	0	0
Bact. cucurbitae	1–320‡	1–2560‡	1–640†	1–160‡	1–2560‡	1–2560‡	1–10240†	1–768§	1–160‡	0†	1–320†	1–320‡	1–320‡	1–80‡	1–160‡	1–160‡	0†	0
Bact. Y	0§	1–160	0	1–80	0	0	0	1–160	1–320	1–1280	0	0	0	0	1–160	0	0	0
Bact. pruni	1–320	1–1280	1–80	1–320	1–80	0	1–80	1–80	1–80	0	1–5120	1–2560	1–160	0	1–320	1–160	0	0
Bact. translucens	1–2560	1–640	1–160	1–160	1–640	1–160	1–80†	1–160†	1–160	0	0	0	1–1280	1–768§	1–2560	1–2560	1–320	0
Bact. translucens var. undulosum	0	1–80	0	0	1–2560	1–320	1–640	1–160	0	0	1–320	1–160	1–768§	1–768§	1–768§	1–768§	1–5120	0
Bact. Z	0	0	0	0	1–320	1–80	0	0	0	0	0	0	1–320	1–2560	1–2560	1–5120	1–2560	0

* Intraperitoneal injection.

† Very slight agglutination in the normal serum, titre 1–80.

‡ Very slight agglutination in the normal serum, titre 1–160.

§ 0, zero indicates no agglutination.

Absorption of agglutinins

It was found that in several instances higher titres were obtained between antisera and heterologous organisms than between antisera and their homologous organisms. Since in such cases absorption of agglutinins has been found to be of use in separating specific and group agglutinins, we decided to make some preliminary absorption tests to determine whether such tests would give promise of applicability in studies of phytopathogenic bacteria. Because the group dispersed we were not able to carry the absorption test far enough in all cases to obtain unequivocal data. The method arbitrarily adopted was that used by Dr. E. O. JORDAN in his studies of the typhoid and paratyphoid group. We are indebted to Miss IRENE YARROW for information relative to the technique. The antisera to be absorbed were made up to a dilution of 1–50 in saline. These antisera were the same as those used in the direct tests. Then separate lots of 5 cc. of the diluted antisera were pipetted into 2-day old agar slants of the organisms used in the absorption. A sterile piece of cotton was pushed with the pipette over the surface, washing the growth down to the bottom of the slant. The mixture of saline and organisms was then filtered through the cotton several times, by pushing the end of the pipette firmly into the cotton, wedging this against the glass, and alternately sucking the mixture into the pipette and expelling it through the cotton. This gave an even suspension free from clumps of bacteria and agar. After this absorption the mixtures were pipetted into centrifuge tubes, incubated for 2 hours at room temperature, and then centrifugalized. Following this the whole procedure was repeated with the absorbed antisera but with new slants. The clear absorbed antisera were then made up in dilutions of 1–150, 1–300, 1–600, 1–1200, and 1–2400, and reciprocal agglutination tests were run with each of the absorbed sera against the homologous and heterologous organisms. Thus, for example, the antisera of *Bact. campestre* and of *Bact. X* absorbed by *Bact. campestre* and the antisera of *Bact. campestre* and of *Bact. X* absorbed by *Bact. X* were tested against suspensions of *Bact. campestre* and *Bact. X*. The mixtures of absorbed antisera and bacterial suspensions were incubated one hour at 37° C., readings were made, and then the mixtures were placed in a refrigerator and readings made after 1 and

12 hours, as in the direct agglutination tests. The final readings are recorded in table II. The absorption test was applied to *Bact. campestre* and *Bact. X*, for which the direct agglutination test had given differentiation to determine whether it would give sharper differentiation; and to *Bact. translucens*, *Bact. translucens* var. *undulosum*,

TABLE II

ABSORPTION TESTS

ANTISERUM OF	TESTED AGAINST SUSPENSION OF	MAXIMUM TITRE	ABSORBED BY SUSPENSION OF	TESTED AGAINST SUSPENSION OF	TITRE (DILUTIONS 1–150 TO 1–2400)	SALINE CONTROL
A: (Bact. campestre)	A	1–10240	A	A	0*	0
	B	1–7680	A	B	1–300	0
			B	A	1–1200	.....
			B	B	0	.....
B: (Bact. X)	A	1–5120	A	A	1–300	.....
	B	1–10240	A	B	0	.....
			B	A	1–2400	.....
			B	B	0	.....
C: (Bact. translucens)	C	1–7680	C	C	0	0
	D	1–7680	C	D	0	0
	E	1–2560	C	E	0	0
			D	C	1–150	.....
			D	D	1–150	.....
			D	E	0	.....
			E	C	1–2400	.....
			E	D	1–2400	.....
			E	E	0	.....
D: (Bact. translucens var. undulosum)	C	1–2560	C	C	1–150	.....
	D	1–7680	C	D	1–150	.....
	E	1–5120	C	E	0	.....
			D	C	1–150	.....
			D	D	0	.....
			D	E	0	.....
			E	C	1–1200	.....
			E	D	1–1200	.....
			E	E	0	.....
E: (Bact. Z)	C	1–320	C	C	1–300	.....
	D	1–5120	C	D	1–300	.....
	E	1–2560	C	E	1–1200	.....
			D	C	1–300	.....
			D	D	1–300	.....
			D	E	1–1200	.....
			E	C	1–300	.....
			E	D	1–600	.....
			E	E	0	.....

*0: Zero indicates no agglutination in dilution 1–150, the lowest used.

and *Bact. Z*, which had not been completely differentiated by the direct agglutination test.

Discussion

In the tests of normal sera slight agglutination occurred in all tubes in which suspensions of *Bact. cucurbitae* were used; however, this never exceeded titres of 1–160. It apparently was due to factors introduced by the contact of sera and antigen, because no agglutination occurred in the saline controls. In two instances very slight agglutination occurred in dilution 1–80 when the normal sera were tested, but not when the antisera were tested (antisera of *Bact. Y* no. 50 and *Bact. Z* no. 48). Very slight agglutination also occurred when the normal sera of animals nos. 30 and 39 were tested against *Bact. translucens* in dilution 1–80; of animal no. 50 against *Bact. X;* of animal no. 41 against *Bact. translucens;* and of animal no. 44 against *Bact. X*. No appreciable difference was noted in the reactions of antisera produced by intravenous and intraperitoneal injection as seen in sera nos. 34 and 33a. Those noted are no greater than the differences obtained with antisera of two animals treated exactly alike, as in the antisera of animals nos. 30 and 39. In the light of our experience, and that of others in the production of antisera with phytopathogenic bacteria, we are of the opinion that experimental work will reveal that intravenous injections give the best titres for some organisms and intraperitoneal injections for others. It seems that dead organisms alone, if they produce any antibodies at all, will not give good titres. It also appears that some organisms when injected while alive are likely to be fatal to the animals. Consequently the safest procedure will be to begin the injections with killed organisms and then change to live organisms in the third injection. Before a final statement can be made a great deal of careful work must be done. This probably will reveal that there is no general rule for phytopathogenic bacteria any more than for other bacteria.

Following the earlier method, we made our final readings of the reactions after 12 hours of refrigeration, during which settling out occurred. In this experiment, in many of the tubes practically no settling out occurred in that time. The flocculi were of the finely granular type rather than of the coarsely granular type previously

encountered in some of the yellow organisms. Whether this difference in the nature of the flocculi in such organisms as *Bact. phaseoli* and *Bact. pruni* indicates that the antisera were less potent than in the earlier experiments, or whether it indicates that changes had occurred in the cultures so that elements which tend to give finely floccular precipitates had come into existence or predominance, we did not have time to determine. *Bact. cucurbitae* flocculated most readily, but this also gave mixtures of the coarse and finely granular type. It is reported, first by WEIL and FELIX (9), ARKWRIGHT (12), and others, that smooth and rough variants of some bacterial colonies give fine and coarse flocculi which settle out slowly and rapidly respectively. The occurrence of a smooth and rough variant in a culture of a phytopathogenic bacterium (*Bact. phaseoli sojense*) has been reported from this laboratory by SHARP (8). There also is evidence that this culture changed. At one time the rough variant of this organism flocculated so easily that it agglutinated spontaneously in distilled water and in saline (8). Later the same organisms gave stable suspensions (4, 5), indicating that its agglutinative properties had changed, possibly because of the origin or the ascendancy of a different element in the culture. If similar changes occurred in the cultures used in these experiments, they were not prominent enough to be detected by change in colony type.[3] Considerable variation was noted in the agglutinability of the organisms tested, *Bact. Y* being least, and *Bact. translucens* var. *undulosum* and *Bact. cucurbitae* most readily agglutinated.

Table I shows that ten of the seventeen antisera gave maximum titres with the homologous organisms, namely, *Bact. campestre*, *Bact. X*, *Bact. cucurbitae*, *Bact. pruni*, and *Bact. translucens* var. *undulosum*. Accordingly the agglutination test can be used to differentiate and identify these organisms among the other yellow organisms tested. The test failed to differentiate for *Bact. phaseoli*, *Bact. Y*, *Bact. translucens*, and *Bact. Z*.

[3] Miss MCCULLOCH has reported (at the time of reading proof) rough variants for *Bact. campestre* and *Bact. campestre* var. *armoraciae* (footnote 2). We had noted uneven appearance in colonies of several of the organisms tested (*Bact. campestre*, *Bact. campestre* var. *armoraciae*, *Bact. cucurbitae*, and *Bact. pruni*), and thought at first that we were dealing with contaminations. This proved not to be the case, but we were unable to segregate smooth and rough variants.

In addition, reciprocal reactions were obtained for the following organisms:[4] *Bact. campestre* and *Bact. X*, *Bact. Y* and *Bact. translucens* var. *undulosum*; *Bact. X* and *Bact. phaseoli* and *Bact. Y*; *Bact. phaseoli* and *Bact. cucurbitae*; *Bact. pruni* and *Bact. translucens* var. *undulosum*; *Bact. cucurbitae and Bact. Y*, *Bact. pruni*, *Bact. translucens*, and *Bact. translucens* var. *undulosum* (all of which are more or less questionable because of reactions with normal sera); *Bact. pruni* and *Bact. translucens* var. *undulosum*; *Bact. translucens* and *Bact. translucens* var. *undulosum* and *Bact. Z*; *Bact. translucens* var. *undulosum* and *Bact. Z*. Of the nine organisms tested, *Bact. campestre* showed reciprocal relationship with three others, *Bact. X* with three, *Bact. phaseoli* with four, *Bact. cucurbitae* definitely with one and possibly with four others, *Bact. Y* with two, *Bact. pruni* with two, *Bact. translucens* with two, *Bact. translucens* var. *undulosum* with five, and *Bact. Z* with two.

In an earlier paper (5) no reaction was reported between antisera of *Bact. campestre* and of *Bact. phaseoli* with *Bact. cucurbitae* and *Bact. pruni* respectively. In the experiments reported here reactions occurred between the antisera of *Bact. campestre* and *Bact. cucurbitae* and suspensions of *Bact. pruni*, and between antisera of *Bact. phaseoli* and *Bact. cucurbitae* and suspensions of *Bact. pruni*. We have no explanation to advance for this discrepancy. The results indicate that the agglutinability of the organisms had increased during the time they were in culture. The reaction of *Bact. phaseoli* displayed another striking deviation from the observations of our earlier experiments. Previously in each case its antiserum had given rather high and maximum titres with the homologous organism. In the experiments reported here its antisera gave lower titres than before, and the maximum titres were given with heterologous organisms. One antiserum (no. 34) gave higher titres with three heterologous organisms (*Bact. cucurbitae*, *Bact. translucens*, and *Bact. translucens* var. *undulosum*) than it did with the homologous organism, while the other antiserum (no. 33a, intraperitoneal injection) gave a higher titre with only one heterologous organism (*Bact. cucurbitae*). This deviation may be due to a change in the

[4] Each reciprocal reaction is listed only once, that is, reciprocal reaction between *Bact. campestre* and *Bact. X*, for example, is listed only under *Bact. campestre*.

culture, possibly because of the origin or the ascendancy of a different element. After these disconcerting results had been obtained we made repeated tests to determine again whether we were in fact working with *Bact. phaseoli*. We found that it was no longer pathogenic to beans, but did find that, according to morphological and physiological tests, it still was *Bact. phaseoli*. Apparently a change in virulence had occurred in the culture. Pathogenicity tests had been made with the culture several months before we made the agglutination tests reported here. It had been found then that the culture was not as virulent as it had been before we made the agglutination tests reported previously (5). If this interpretation of the data is correct, we have an instance of change in virulence of a culture and a parallel change in serological reaction.

It appears therefore that more or less relationship exists between the organisms tested here of the yellow pigmented type represented by *Bact. campestre*. The conclusion that these organisms show group reaction is in part in apparent contradiction with the conclusion of BROOKS, NAIN, and RHODES (3), that no relationship exists between *Bact. pruni* and other yellow organisms tested by them, such as *Bact. campestre* and *Bact. phaseoli*, and also with our earlier tentative conclusion, based on tests which were not reciprocal, in which we concurred with the conclusion of BROOKS and associates (5). It should be borne in mind, however, that these experimenters based their conclusions on data obtained with antisera in dilutions of 1–100, whereas we have incorporated into the summary data obtained with dilutions of 1–80. If we do not consider these titres then we must strike from our list of reciprocal reactions those between *Bact. campestre* and *Bact. translucens* var. *undulosum;* of *Bact. X* and *Bact Y;* of *Bact. phaseoli* and *Bact. pruni* and *Bact. cucurbitae;* and of *Bact. cucurbitae* and *Bact. pruni*. This would lead to the same conclusion relative to *Bact. pruni* as that of BROOKS, NAIN, and RHODES (3).

While the data indicate that more or less serological relationship exists between those bacteria with yellow pigment which morphologically and culturally seem to be closely related, the serological tests have not revealed whether there is any one parent form. In our experiments *Bact. translucens* var. *undulosum* showed more extensive

relationship than any other organism. This may be due in part to its ready agglutinability. Theoretically, if one had at one's disposal all the extant organisms of this group and could make the serological test quite quantitatively, it might throw some light on the relationship of not only these organisms and their derivation but also possibly on the relationship of their hosts. It is interesting to note in this connection that of the three organisms sent us by Miss McCulloch, which are morphologically and culturally scarcely distinguishable from *Bact. campestre*, *Bact. X* (which attacks a member of the Cruciferae) is the most closely related serologically to *Bact. campestre* (which also attacks Cruciferae); and that *Bact. translucens*, *Bact. translucens* var. *undulosum*, and *Bact. Z* (which attack cereals) are more closely related to one another than to any other of the organisms tested.

In the light of the report of Miss McCulloch relative to the relationship of *Bact. campestre*, *Bact. X*, *Bact. cucurbitae*, and *Bact. Y*, it is interesting to note that serologically these organisms show varying group reaction. Although *Bact. campestre* and *Bact. X* show very strong group reaction, differentiation nevertheless was possible by dilution of antisera in the agglutination test.

In order to put the question of the relationship of *Bact. campestre* and *Bact. X* to a more severe analysis, the absorption test was applied. Although the results are not absolutely clear-cut, they indicate that while *Bact. campestre* and *Bact. X* are very closely related they are distinct antigenically. That they are closely related is indicated by the fact that high degrees of absorption are obtained when the antisera of both organisms are absorbed by homologous and heterologous organisms. That they are not quite identical is indicated by two facts. On the one hand, absorption of the antiserum of *Bact. campestre* by *Bact. campestre* so completely removed the specific agglutinins that *Bact. campestre* was not agglutinated by this absorbed antiserum in dilution 1–150, and also removed group agglutinins to such an extent that *Bact. X* was not agglutinated by this absorbed antiserum in dilutions above 1–300. On the other hand, absorption of this antiserum by *Bact. X* so completely removed the group agglutinins that *Bact. X* was not agglutinated by it in dilution 1–150, whereas the specific agglutinins were reduced but

gave a titre of 1–1200 when this absorbed antiserum was tested against *Bact. campestre*. The tests with the absorbed antisera of *Bact. X* indicate that the organisms are related, but they do not show whether they are distinct. We were not able to pursue this study further to determine the significance of the titres obtained. The titre of 1–300 obtained when the antiserum of *Bact. X* absorbed by *Bact. campestre* was tested against *Bact. campestre*, probably indicates that absorption was not complete enough to remove the agglutinins.

Bact. cucurbitae apparently is related to both *Bact. campestre* and *Bact. X*, but is less closely related to them than they are to each other. The test also clearly differentiated *Bact. cucurbitae* from *Bact. Y*. *Bact. Y* apparently is related to *Bact. X* but not very closely; it is more closely related to *Bact. campestre*. In fact, the antiserum of *Bact. Y* gave a higher titre with *Bact. campestre* than with the homologous organisms, which may indicate a very close relationship. The higher titre may be due to greater agglutinability on the part of *Bact. campestre*. Unfortunately we did not have time to make absorption tests. Even though the agglutination tests failed to differentiate *Bact. campestre* and *Bact. Y* when the antiserum of *Bact. Y* was used, the serological reactions of the organisms taken as a whole indicate that they differ antigenically. With the exception of *Bact. Y*, these organisms show some relationship to *Bact. phaseoli*. With the possible exception of *Bact. cucurbitae*, their relationship to *Bact. pruni* is very slight. Their relationship to the cereal group also is slight. It appears, then, that though these organisms (*Bact. campestre*, *Bact. X*, *Bact. cucurbitae*, and *Bact. Y*) are antigenically related to form a loose group of varying relationship among one another, and show varying individual relationship to *Bact. phaseoli*, *Bact. pruni*, and the cereal group, they are sufficiently distinct antigenically to permit differentiation by the agglutination test.

When we consider the data relative to *Bact. translucens*, *Bact. translucens* var. *undulosum*, and *Bact. Z*, a very close group reaction is found. The relationship is so strong that the direct agglutination test did not differentiate between *Bact. translucens* and *Bact. translucens* var. *undulosum* when the antiserum of *Bact. translucens* was

used, nor between *Bact. Z* and *Bact. translucens* var. *undulosum* when the antiserum of *Bact. Z* was used. It may be significant in this connection, that *Bact. translucens* var. *undulosum* agglutinates very readily. The agglutination test did differentiate between *Bact. translucens* and *Bact. Z*, and between *Bact. translucens* and *Bact. translucens* var. *undulosum*, when the antisera of *Bact. translucens* var. *undulosum* were used.

The absorption test showed that these organisms are closely related, *Bact. Z* being the least closely related. The test showed clearly that *Bact. Z* is distinct antigenically. It did not differentiate between *Bact. translucens* and *Bact. translucens* var. *undulosum*. Whether this indicates that the absorption test will not differentiate between them or whether more work would have cleared up the situation we do not know. It is apparent from the data that we did not completely absorb when we absorbed the antiserum of *Bact. translucens* with *Bact. translucens* var. *undulosum*, the antiserum of *Bact. translucens* var. *undulosum* with *Bact. translucens*, and the antiserum of *Bact. Z* with *Bact. translucens* and *Bact. translucens* var. *undulosum*. Only *Bact. translucens* and *Bact. translucens* var. *undulosum* show some relationship to the other yellow organisms tested. This is most pronounced with *Bact. phaseoli*, *Bact. cucurbitae*, and *Bact. campestre*, and very slight with *Bact. pruni* and *Bact. Y*. If one considers all the antigenic reactions of the cereal organisms, it is apparent that they are closely related but distinct antigenically.

Summary

1. *Bact. campestre*, *Bact. X*, *Bact. cucurbitae*, *Bact. pruni*, and *Bact. translucens* var. *undulosum* were differentiated by direct agglutination tests with their antisera.

2. Cross agglutination so high that differentiation by the direct agglutination test was not possible occurred between antisera of *Bact. phaseoli* and the heterologous organisms *Bact. X*, *Bact. cucurbitae*, *Bact. translucens*, *Bact. translucens* var. *undulosum*, and *Bact. Z;* between the antisera of *Bact. Y*, and *Bact. campestre;* between the antisera of *Bact. translucens*, and *Bact. translucens* var. *undulosum;* and between the antisera of *Bact. Z*, and *Bact. translucens* var. *undulosum*.

3. Absorption of agglutinins was carried on with the cereal pathogens, and differentiated *Bact. Z* from the others; but, probably because of incomplete absorption, did not differentiate between *Bact. translucens* and *Bact. translucens* var. *undulosum*.

4. Even though the direct agglutination test did not differentiate all the organisms tested, consideration of all the reciprocal reactions of these organisms warrants the tentative conclusion that they are all distinct antigenically.

5. If we consider titres in dilutions of 1–80, group reaction of varying degree was obtained for all the organisms tested, namely, *Bact. campestre*, *Bact. X* (horse radish pathogen), *Bact. phaseoli*, *Bact. cucurbitae*, *Bact. Y* (poppy pathogen), *Bact. pruni*, *Bact. translucens*, *Bact. translucens* var. *undulosum*, and *Bact. Z* (cereal pathogen). *Bact. Z*, *Bact. pruni*, and *Bact. Y* gave the slightest group reactions, in the order named.

6. *Bact. campestre*, *Bact. X*, *Bact. cucurbitae*, and *Bact. Y* antigenically constitute a loose group of varying interrelationship and of varying relationship to the other yellow organisms tested. *Bact. campestre* is very closely related to *Bact. X* and to *Bact. Y*. With the possible exception of *Bact. cucurbitae*, these four organisms are not closely related to *Bact. pruni*, and with the exception of *Bact. Y* they show some relationship to *Bact. phaseoli*. Their relationship to the cereal group is slight.

7. The cereal pathogens *Bact. translucens*, *Bact. translucens* var. *undulosum*, and *Bact. Z* antigenically constitute a closely related group, with varying relationship to the other organisms tested. *Bact. Z* shows the least relationship and *Bact. translucens* var. *undulosum* the most extensive. This relationship is strongest with *Bact. phaseoli*, *Bact. cucurbitae*, and *Bact. campestre*, and slightest with *Bact. pruni* and *Bact. Y*.

8. The exploratory studies of this and the antecedent papers indicate that the agglutination test may find a use in phytobacteriology analogous to its rôle in other fields of bacteriological study. For some organisms the direct agglutination test apparently can be developed to serve in identification of species and strains; for others, absorption of agglutinins will have to be resorted to.

9. These studies also give promise that the agglutination and

absorption of agglutinin tests can be used in grouping and classification of at least some closely related species, varieties, or subvarieties of phytopathogenic bacteria. Before these tests can lead to unequivocal conclusions, even for the group which we have studied, however, considerable more detailed and penetrating work than we have undertaken must be done. In other words, the experimental procedure and the applicability of each method must be determined in reproducible detail for each species and subspecies, and for such groups into which they may fall. Cognizance will have to be taken of such factors as correct titres, varying degrees of agglutinability, spontaneous agglutination, different types of flocculi, etc.

10. Fully as interesting and significant as the few clarifying results here obtained, are the very observations which have introduced factors of apparent uncertainty into some of our experiments. Among these the most significant are the evidences of variation which we have obtained. In addition to the variant of the rough-smooth type (**4, 8**), the group shows other types of variation whose precise nature was not determined (behavior of *Bact. phaseoli* and others). Study of this phenomenon of variation promises to become an interesting field in itself, in which serological methods may prove as useful as they have in the study of variants in bacteria other than plant pathogens.

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MATROCLINY IN FLOWER SIZE IN RECIPROCAL F_1 HYBRIDS BETWEEN DIGITALIS LUTEA AND DIGITALIS PURPUREA[1]

J. Ben Hill

(with three figures)

Introduction

Many hybridization experiments have been conducted between *Digitalis purpurea* L. and *Digitalis lutea* L. Kölreuter (5), Gärtner (2), and Focke (1) all worked with this hybrid combination. Kölreuter secured F_1 hybrids of *D. purpurea*×*D. lutea* but failed to secure the reciprocal, although he secured reciprocal F_1 hybrids of the *D. lutea-D. thapsi* combination. If *D. thapsi* is actually a distinct species, it is a very close relative of *D. purpurea*. Gärtner and Focke both secured reciprocal F_1 hybrids of the *D. purpurea-D. lutea* combination. Gärtner observed two types of plants in the F_1 reciprocal hybrids of the *D. purpurea-D lutea* combination, one type with large yellow flowers and another with flowers differing only slightly from those of *D. lutea*, excepting that they had a reddish tinge. Earlier, Kölreuter (5) had observed variations in the F_1 generation of *D. purpurea*×*D. lutea*, and also unlike reciprocal F_1 hybrids between other *Digitalis* species. Focke, who summarized the literature on the topic to 1881, found that reciprocal F_1 hybrids between *D. purpurea* and *D. lutea* differ in flower color, F_1 plants of *D. purpurea*×*D. lutea* bearing flowers of a more beautiful color than the reciprocal. He states that Kölreuter's and Gärtner's observations of differences in reciprocals would bear further investigation. Due to the facts that the number of individuals was often small, that very superficial characters (such as slight differences in corolla coloration) were often the basis of comparisons, and that descriptions of so-called natural hybrids of uncertain parentage were fre-

[1] Contribution from the Department of Botany, The Pennsylvania State College, no. 63, published with the approval of the Director of the Agricultural Experiment Station as a partial report on project no. 657. Technical paper no. 454.

quently given undeserved consideration in the descriptions, the earlier accounts are unsatisfactory.

WILSON (9) reported unlike reciprocals with matroclinous tendencies in the F_1 hybrids between *D. purpurea* and *D. lutea*. He reported that the F_1 hybrid plants produce flowers differing as to form and size in the reciprocals, and resembling those of the seed parent to a greater degree than those of the pollen parent. His discussion is primarily of infertile hybrids, and the matter of unlike reciprocals and matrocliny scarcely receives the attention that the subject deserves. WILSON was correct in his observation of unlike reciprocal F_1 hybrids between these species as regards flower size. His discussion of differences of corolla color in the reciprocal hybrids is somewhat misleading, since the observed differences were due to the segregation of corolla color factors in the gametes of the *D. purpurea* parent. *D. purpurea* is generally heterozygous for color factors. When such heterozygous plants are crossed with those of another species bearing yellow flowers, as *D. lutea* or *D. ambigua*, the F_1 hybrids of the heterozygous parents frequently vary among themselves considerably, due to the distribution of the various color factors to the F_1 zygotes.

JONES (4) gives a summary of the literature on species crosses in *Digitalis*, including the *D. purpurea-D. lutea* combinations. WARREN (7, 8) has recorded his results on crosses between *D. purpurea* and *D. lutea*. He reported observations on the reciprocal crosses between the parental species, and especially the results of backcrossing the F_1 hybrids to the parental species. He reported fertility of F_1 hybrids when these plants were backcrossed to the parental strains. These results are especially interesting in view of the fact that all previous investigators report total sterility of the F_1 hybrids between *D. purpurea* and *D. lutea*. I have grown several hundred F_1 plants of the *D. purpurea-D. lutea* combination, using several strains of both parents, and have never observed the slightest evidence of fertility in any F_1 hybrid of this combination; in fact, I have never seen a fertile F_1 hybrid in which *D. purpurea* was one of the parents, even though species other than *D. lutea* were used in the crosses. WARREN admitted that there was some question as to the taxonomic position of the *D. lutea* which he used in his experiments, and stated that it

was the opinion of Dr. ARTHUR A. HILL of the Kew Gardens that this particular strain is a fertile hybrid between *D. ambigua* and *D. lutea*. The published photographs of WARREN'S *D. lutea* indicate that these plants are very much like a certain small-flowered *D. ambigua* which I received from the Edinburgh Botanic Gardens.

In a recent paper (3) on cotyledon form and size, it was stated that the cotyledons exhibited the only evidence of matrocliny which I had observed in F_1 hybrids between any species of *Digitalis*. The parent species *D. purpurea* and *D. lutea* differ markedly in the shape and size of the cotyledons, and their reciprocal F_1 hybrids show distinct matroclinous tendencies in respect to cotyledon form and size. Since gathering the data for that paper, however, I have grown great numbers of several pairs of reciprocal F_1 hybrids between various strains of *D. purpurea* and *D. lutea*, and now wish to report my observations of matrocliny in flower size in these hybrids.

Investigation

D. lutea bears small yellow flowers whose corollas average about 2.112 cm. in length and 0.619 in width. The numerous strains of *D. lutea*[2] vary slightly in flower size. The varieties of *D. purpurea* vary in flower color from pure white, white with spots of various colors, to various shades of red, lavender, and purple. The size of the corolla of an inbred strain (45) of *D. purpurea* used in some of the experiments was an average of 4.936 cm. in length and 2.2 cm. in width. Other strains of this species may vary in size from these measurements, but this size is fairly typical of all varieties of the species.

The corolla color of the flowers of the F_1 hybrid between *D. lutea* and *D. purpurea* varies, depending upon the particular colors of the *D. purpurea* parent. If this parent is a white variety without anthocyanin even in the spots in the corolla, the F_1 hybrids are white, the yellow coloration being recessive. If the *D. purpurea* parent is a variety bearing flowers with any degree of coloration, however, the F_1 hybrids will bear flowers with corollas of some shade of purple or

[2] I wish to acknowledge my indebtedness to the Botanic Gardens of Kew, Dublin, Edinburgh, and Cambridge; the Museum d'histoire Naturelle, Paris; and especially the Jardin Botanico, Madrid, for the collections of seeds of *Digitalis* species used in this study of the hybrids.

red. The degree of color may vary greatly, depending upon the interaction of the genes for corolla coloration.

The reciprocal F_1 hybrid plants of the *D. lutea-D. purpurea* combination are indistinguishable in the rosette stage, and bear inflorescences which in general form are identical in appearance (fig. 1). The F_1 hybrids of this combination bear flowers, however,

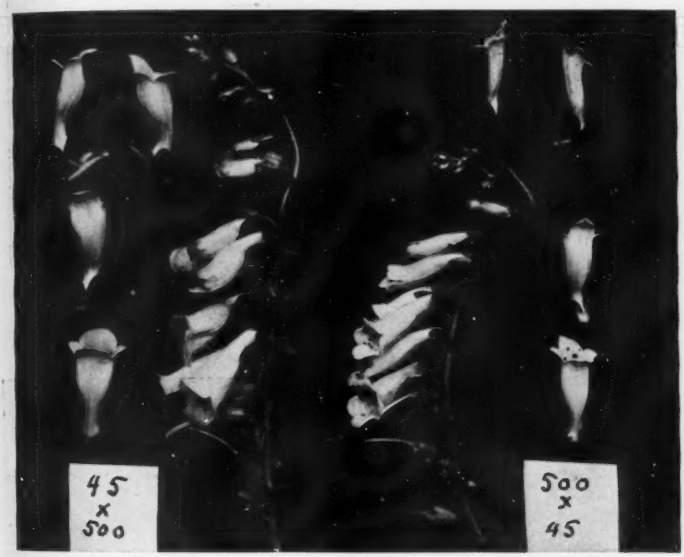

FIG. 1.—*D. purpurea* (45)×*D. lutea* (500), and *D. lutea* (500)×*D. purpurea* (45); inflorescences from reciprocal F_1 hybrid plants.

which differ markedly in size in the reciprocals, and exhibit a distinct matrocliny, resembling the female parent decidedly more than they do the male parent (figs. 2, 3). Measurements[3] show that these matroclinous tendencies include both corolla and sepal size (tables I, II). The flower parts differ especially in width.

The most significant data used in this report were taken on hybrid combinations between two varieties of *D. purpurea* and two

[3] I wish to give acknowledgments of assistance by Mrs. HELEN D. HILL, Mrs. EDITH HOLMES, and Miss FLORENCE BROWN in accumulating the data herewith presented.

strains of *D. lutea* secured from different sources. An inbred white variety of *D. purpurea* is designated by the number 45, and an inbred

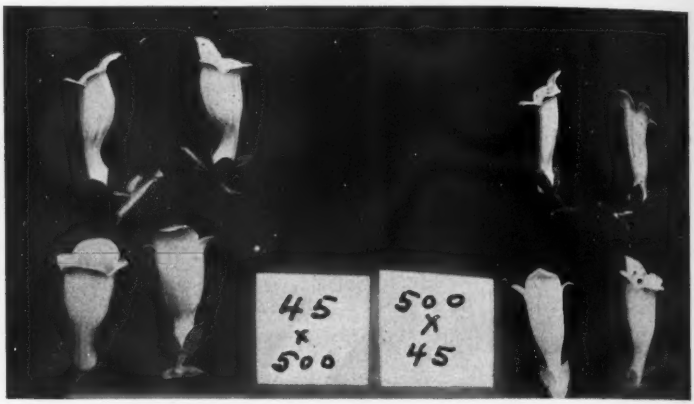

FIG. 2.—*D. purpurea* (45)×*D. lutea* (500), and *D. lutea* (500)×*D. purpurea* (45); individual flowers from F_1 hybrid plants.

FIG. 3.—*D. lutea* (530)×*D. purpurea* (960), and *D. purpurea* (960)×*D. lutea* (530); individual flowers from F_1 hybrid plants.

dark purple variety of the same species bears the number 960. The *D. lutea* strains are designated by the numbers 500 and 530, the latter one having been secured from the Botanic Garden of Edin-

burgh. Other varieties and strains were used in the investigation, and data from the results are incorporated in this report; but reciprocal hybrids involving these strains have yielded the most definite evidence of unlike reciprocals and matrocliny.

TABLE I

COROLLA AND SEPAL LENGTH AND WIDTH IN PARENT SPECIES AND RECIPROCAL HYBRIDS

	SPECIES AND RECIPROCAL HYBRIDS					
	D. LUTEA (535)	D. LUTEA (530) × D. PURPUREA (960)	D. PURPUREA (960) × D. LUTEA (530)	D. LUTEA (500) × D. PURPUREA (960)	D. PURPUREA (960) × D. LUTEA (500)	D. PURPUREA (45)
	Number of measurements (cm.)					
	Corollas 25, sepals 25	Corollas 25, sepals 25	Corollas 25, sepals 25	Corollas 38, sepals 25	Corollas 45, sepals 25	Corollas 25, sepals 25
Corolla						
Average length	1.995	2.96	3.488	2.961	3.152	4.936
Average width	0.577	0.83	1.15	0.754	1.062	2.2
Calyx						
First sepal (smallest)						
Average length	0.386	0.43	.54	.53	0.59	1.15
Average width	0.120	.14	.17	.10	.13	.47
Second sepal						
Average length	0.478	.56	.66	.60	.69	1.44
Average width	0.110	.19	.21	.16	.20	.77
Third sepal						
Average length	0.464	.53	.60	.63	.68	1.14
Average width	0.208	.27	.28	.22	.27	.87
Fourth sepal						
Average length	0.484	.53	.59	.61	.67	1.36
Average width	0.191	.27	.28	.21	.26	.88
Fifth sepal						
Average length	0.462	.56	.64	.60	.67	1.38
Average width	0.118	0.19	0.21	0.16	0.19	0.76

The difference and the matroclinous tendencies in flower size in the reciprocal F_1 hybrid plants are well illustrated in the case of *D. lutea* (530)×*D. purpurea* (960). The difference is clearly shown in fig. 3, and in the measurements of flower sizes in reciprocals (tables I, II). The hybrid *D. lutea* (530)×*D. purpurea* (960), whose seed parent is the smaller-flowered *D. lutea*, bears flowers which are dis-

tinctly smaller than those of the hybrid *D. purpurea* (960) × *D. lutea* (530), whose seed parent is the larger-flowered *D. purpurea*. The average measurements of corolla length are 2.96 cm. for the former and 3.488 cm. for the latter, and of corolla width 0.83 cm. and 1.15 cm. respectively (table I). *D. purpurea* (960) × *D. lutea* (530) bears

TABLE II

PERCENTAGE DIFFERENCES OF COROLLA AND SEPAL SIZE IN RECIPROCAL HYBRIDS

RECIPROCAL HYBRIDS COMPARED	NO. OF MEASUREMENTS	PERCENTAGE DIFFERENCE											
		COROLLA		CALYX									
				FIRST SEPAL (SMALLEST)		SECOND SEPAL		THIRD SEPAL		FOURTH SEPAL		FIFTH SEPAL	
		Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
D. purpurea (960) × D. lutea (500)	25*	6.50	40.84	11.32	30.0	15.0	25.0	7.94	22.73	9.83	23.81	11.66	18.75
compared with													
D. lutea (500) × D. purpurea (960)	25†												
D. purpurea (960) × D. lutea (530)	25	17.83	38.50	25.58	21.42	17.85	10.52	13.20	3.70	11.32	3.70	14.29	10.52
compared with													
D. lutea (530) × D. purpurea	25												
Total of all D. purpurea × D. lutea	50‡	8.96	43.59	10.66	37.49	8.15	2.90	5.07	24.73	6.23	−13.17	9.50	− 0.18
compared with													
Total of all D. lutea × D. purpurea	164												

* Corollas 45. † Corollas 38. ‡ Corollas 70.

flowers which are 17.83 per cent longer and 38.5 per cent broader than the reciprocal (table II).

D. purpurea (960), in combination with another strain of *D. lutea* (indicated by the number 500), produced F_1 hybrids bearing flowers with the same matroclinous tendencies and to about the same degree; that is, 6.5 per cent difference in length and 40.84 per cent difference in width of corolla (table II).

Similar differences, also with matroclinous tendencies, may be seen in the measurements of sepal length and width (tables I, II). The sepals were measured, designating the smallest sepal as the first and the others in rotation as second, third, fourth, and fifth respectively. As in the corolla size, there is a greater difference in width than in length of sepal in the flowers of these particular hybrid combinations.

The totals of all measurements secured for flower size in the *D. lutea-D. purpurea* F_1 reciprocal hybrids, when grouped for comparison (table II), show a difference for corolla length of 8.96 per cent and for corolla width of 43.59 per cent. These differences in both instances indicate matrocliny. Certain of the sepal measurements for the total group fail to indicate matrocliny and are designated by a minus sign.

Summary

Reciprocal F_1 hybrids between *Digitalis lutea* and *D. purpurea* are unlike and matroclinous in respect to flower size. These differences appear in both corolla and calyx.

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4. Jones, W. N., Species hybrids of *Digitalis*. Jour. Genetics **2**:71–88. 1912–1913.
5. Kölreuter, J. G., Digitales hybridae. Acta Acad. Imperialis Scientarum. Petrop. pars. **1**:215–233. 1777.
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BRIEFER ARTICLES

THREE NEW DINOFLAGELLATES FROM NEW JERSEY[1]

(WITH TWELVE FIGURES)

The dinoflagellates from brackish waters of New Jersey, herewith described, have been observed in connection with studies on the food of oysters which have been in progress for some years. *Prorocentrum triangulatum* is certainly, and *Amphidinium fusiforme* is probably of great importance in this connection. *Polykrikos barnegatensis* is evidently rare, but represents an interesting addition to a curious and not too well known genus.

Prorocentrum triangulatum sp. nov.

Cell strongly compressed, triangular as seen from side, rounded behind, left valve with a delicate tooth. Surface of valves covered with minute poroids, the margins appearing striate. Chromatophores yellow-brown, irregular, commonly broken up into rather small masses. Length (without tooth) 17–22 μ.

Extremely common in Barnegat Bay, where it is sometimes the most abundant organism in oysters' stomachs; also in Delaware Bay. This is obviously one of the thirteen organisms listed by GRAVE[2] as forming the bulk of the food of Chesapeake Bay oysters. He illustrates it but gives it no name. It is difficult to believe that so common and abundant a form as this has been undescribed, but I cannot place it in any recorded species. Dr. MARIE LEBOUR, who has been kind enough to examine the description and drawings, also expresses the opinion that it is undescribed. I venture, therefore, to describe it as a new species.

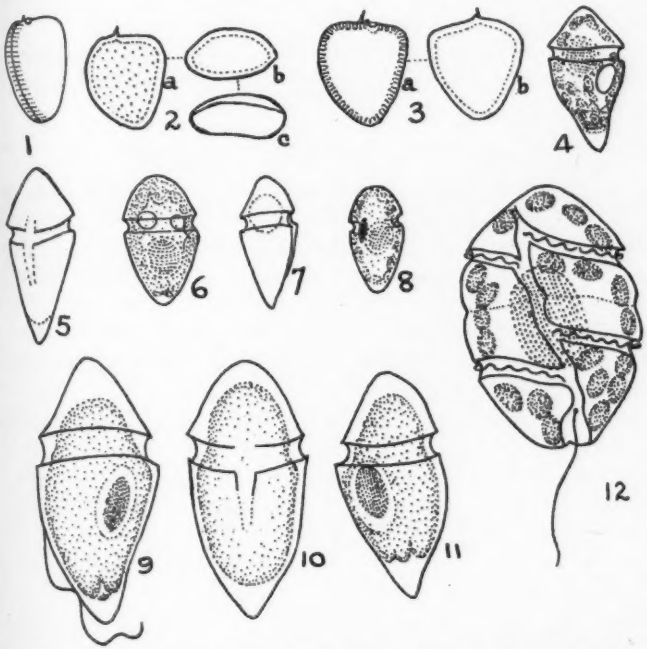
Amphidinium fusiforme sp. nov.

Cell fusiform, about twice as long as broad, circular in cross-section. Epicone hemispherical-conical; girdle anterior; hypocone two to two and one-half times as long as the epicone, long conical or somewhat helmet-shaped; sulcus obscure, but apparently encroaching upon the epicone

[1] Publication no. 15, New Jersey Oyster Investigation Laboratory. Paper of the Journal Series, New Jersey Agricultural Experiment Station.

[2] GRAVE, C., Fourth report of the shell-fish commission of Maryland. pp. 324–348. Baltimore. 1912.

and extending at least half way from the girdle to the antapex. Body packed with yellowish green chromatophores except at the posterior end, which is hyaline; a dull orange stigma is visible in ventral region beneath



FIGS. 1–12.—Figs. 1–3, *Procentrum triangulatum:* fig. 1, right dorsal; fig. 2 (*a*) right lateral, (*b*) optical section from anterior end, (*c*) posterior view of same individual; fig. 3, right and left sides of same individual; ×1110. Figs. 4–11, *Amphidinium fusiforme:* fig. 4, right lateral, from life, ×1060; fig. 5, ventral, from life, ×1060; fig. 6, dorsal, from life, ×1060; fig. 7, right lateral showing sulcus, killed in strong IKI and preserved in formalin, ×1110; fig. 8, ventral, from life, showing stigma, ×1060; figs. 9–11, left lateral, ventral and right dorsal respectively, killed in osmic acid, followed by chrom-acetic and preserved in formalin (plasmolysis of contents permits clear view of delicate theca), ×2436; fig. 12, *Polykrikos barnegatensis:* ventral, from living specimen (anterior longitudinal flagellum not seen), ×1060. All figures drawn with camera lucida at magnifications indicated and reduced one-fifth in reproduction.

the girdle. Nucleus central and dorsal, mostly behind the girdle. The entire body incased in a delicate pellicle, usually visible only in fixed specimens in which the contents of the cells have become plasmolyzed.

Length usually 17–22 μ; diameter 8–11 μ; sometimes larger or smaller. The smallest individual measured was 12.6×7.3, the largest 31×13.6 μ.

In shape, size, structure, and the possession of a pellicle, close to *A. crassum* Lohmann, but smaller, proportionately narrower and more fusoid, and possessing chromatophores.

Barnegat Bay, common; Delaware Bay, exceedingly abundant locally, occurring in dense swarms in late summer and giving rise to the phenomenon of "red water."

Polykrikos barnegatensis sp. nov.

Body ovate, nearly circular in cross-section, slightly concave on ventral side, composed of two zooids, closely united, with a very slight constriction between the individual zooids and a single large beaded nucleus in center; girdle or each zooid a descending left spiral, displaced twice its width; sulcus extending from just below the anterior end to the posterior end, which is slightly 2-lobed. Ground protoplasm colorless, but containing numerous oval, yellow-brown chromatophores. Nematocysts lacking; evidently autophytic. Length 46 μ; diameter 31.5 μ.

Barnegat Bay, September 7, 1928. But one individual seen, which was observed in living condition for nearly half an hour, when it underwent cytolysis.—G. W. MARTIN, *State University of Iowa, Iowa City.*

[*Accepted for publication November 2, 1928*]

CURRENT LITERATURE

BOOK REVIEWS

Tree books

The increasing interest of the public in the larger woody plants of the country is shown by the increasing number of books on trees and shrubs. It seems to be the ambition of every state to have a tree book of its own, and Minnesota[1] and Kansas[2] have just issued their volumes.

The former is the more pretentious, taking the form of an illustrated volume and including shrubs as well as trees. Its interpretation of terms is so liberal as to include cultivated as well as native trees, and to extend the idea of shrubs to such half woody forms as species of *Spiraea* and *Ceanothus*. Its illustrations consist of good drawings of the leaves and flowers of many of the species described. Perhaps its most notable features are the number and elaborateness of its keys. These should make the identification of species a comparatively easy matter.

The Kansas book appears more modestly, in paper covers, but contains almost as many pages, more illustrations, and quite as much subject matter. A third of the volume is devoted to economic uses of trees, and is illustrated by numerous photographs, which include tree habits, tree trunks, and tree industries. In the second part is a manual of trees, illustrated by drawings already familiar to us in *Michigan trees*. It includes many species, like white pine and Douglas fir, that are not native to Kansas, indeed are scarcely planted within its borders. It is well suited to its purpose of increasing interest in tree study and tree planting.—G. D. FULLER.

The problem of fermentation

A little monograph on alcoholic fermentation, written two years ago by SCHOEN[3] of the Institute Pasteur, has just been issued in English translation. A chapter on the rôle of hydrogen in biological oxidation has been added especially for this edition, and is the clearest statement of the nature of cell oxidations which the reviewer has seen. The whole monograph is characterized

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[1] ROSENDAHL, C. O., and BUTTERS, F. K., Trees and shrubs of Minnesota. 8vo. pp. 385. *figs. 146*. Univ. of Minn. Press, Minneapolis. 1928. $4.

[2] SCOTT, C. A., and GATES, F. C., Trees in Kansas. 12mo. pp. 372. *figs. 209*. Report, Kansas State Board of Agric., J. C. MOHLER Secretary. Topeka, Kansas. 1928.

[3] SCHOEN, M., The problem of fermentation. The facts and the hypotheses. Transl. by HIND, L. H. pp. xii+210. London: Chapman and Hall. 1928.

by unusual lucidity, and is an admirable summary of the present status of our knowledge of fermentation by one who has worked at the problem for many years. There is a good bibliography.—H. S. WOLFE.

NOTES FOR STUDENTS

Peat bogs and postglacial vegetation.—Most of our forest trees produce an abundance of pollen. This wind-borne pollen seems to be rather evenly distributed over forested areas, and falling into lakes and bogs it is there preserved on account of its resistant exine, and may be recognized in lake and peat deposits thousands of years later. The relative amounts of such fossilized pollens from different tree species are believed to give a rather accurate picture of the forest vegetation of the area at the time deposits were being laid down. Because of this hypothesis there has developed an active study of peat deposits by means of pollen analyses. Its beginning in Sweden early in the present century and its spread into various other European countries has already been noted in this journal.[4] More recently similar studies have been undertaken in America.

Working with assistance afforded by the International Education Board and the Geological Survey of Canada, AUER[5] has made a survey of some thirty-four bogs in southeastern Canada, ranging in their distribution from Nova Scotia to Niagara, Ontario. He differentiates bogs of oceanic and continental types dependent on existing climatic conditions. The bogs of the oceanic type are found principally in Nova Scotia and New Brunswick, under the influence of high atmospheric humidity. They seldom result from the filling up of lakes; they have frequently a convex surface; they are dominated by *Sphagnum;* and have usually very uniform peat deposits. In many instances their lower strata show marine deposits. The bogs of the continental type, on the contrary, very commonly come from the filling up of lakes or ponds; they have flat or concave surfaces; they are largely dominated by *Carex*, with *Amblystegium* formerly very important; and their peat deposits lack the uniformity of oceanic bogs. Various transitional forms are distinguished, connecting the two main types.

AUER's classification of peat deposits, while not entirely new, is logical and useful. The four classes are: (1) Limnistic peat, that might also be called lake peat, consisting of rather amorphic ooze that may be either inorganic or organic. The former may contain traces of plankton while the latter is made up largely of remains of Diatomaceae and Desmidiaceae. In addition, there is at times a detritus ooze containing numerous remains of such submerged aquatics as *Ceratophyllum*, *Myriophyllum*, and *Naias*. This peat frequently occurs as lens-shaped deposits near the former shores of filled lakes. (2) Telmatic or marsh peat, which is a form in which *Carex* is the chief constituent and which is found

[4] BOT. GAZ. 83:323–325. 1927.

[5] AUER, VÄINÖ, Stratigraphical and morphological investigations of peat bogs of southeastern Canada. Communicationes. Inst. Quaest. Froestalium Finlandiae Editae 12:1–62. 1927.

as a thinner or thicker layer in almost all bogs. It is particularly abundant in bog deposits of the continental type. This carex peat may be either quite pure or may contain mixtures of the remains of such plants as *Eriophorum vaginatum*, *Amblystegium*, or *Sphagnum*. (3) Semi-terrestic peat; this is essentially sphagnum peat often forming thick strata that are exceptionally pure and homogeneous. (4) Terrestic peat; this is deposited from bog forests and consists of sphagnum peat together with tree stumps and the remains of trees and shrubs.

With respect to the nature of the stratified deposits, AUER distinguishes three types of bogs. They are sphagnum peat bogs, composed of semi-terrestic or terrestic peat; carex peat bogs, with deposits of telmatic peat; and a combination of these in which different peats are variously stratified.

AUER'S investigations were conducted with the usual technique of peat boring and pollen analysis. The various bogs visited are described in detail as to their appearance and stratification. Diagrammatic cross-sections of profiles are given for eight of the more important, with pollen diagrams for twelve. Variations are found that are widespread, and show distinctly warmer and cooler periods which are synchronous throughout the area studied. The changes are similar to those that have obtained in Europe, and the conclusion is that the BLYTT-SERNANDER classification of postglacial periods may also be applied in Canada. The evidence from this investigation seems to show that an early, dry, warm, boreal period was followed by a well marked atlantic period with moist warm climate. This was followed in turn by a drier sub-boreal period and a cooler subatlantic period, both rather poorly marked. The further study of AUER'S material may serve to clear up many doubtful points and emphasize the need of further investigations.

ERDTMAN, who has been one of the most active workers in this field, has recently brought together the extensive literature on the subject,[6] together with a plate illustrating the principal tree pollens and a map showing where pollen analyses have been carried out in Europe. An extensive atlas and key to all pollens likely to be found in bogs has been compiled by MEINKE,[7] and should be of material assistance to workers in this field.

ERDTMAN has also been extending his investigations in the British Isles,[8] in Holland,[9] and in Belgium and France,[10] confirming results that were formerly

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[6] ERDTMAN, G., Literature on pollen-statistics published before 1927. Geolog. Fören. Stockholm Förhrandl. **49**:196–211. 1927.

[7] MEINKE, HERBERT, Atlas und Bestimmungsschlüssel zur Pollenanalytik. Bot. Archiv. **19**:380–449. 1927.

[8] ERDTMAN, G., Studies in the postarctic history of the forests of northwestern Europe. I. Investigations in the British Isles. Geolog. Fören. Stockholm Förhandl. **50**:123–192. 1928.

[9] ———, Studien über die postarctische Geschichte der nordwesteuropäischen Wälder. II. Untersuchungen in Nordwestdeutschland und Holland. *Ibid.* **50**:368–379. 1928.

[10] ———, Études sur l'histoire postarctique des forêts de l'Europe Nord-ouest. III. Recherches dans la Belgique et au Nord de la France. *Ibid.* **50**:419–428. 1928.

announced, while RUDOLPH[11] has summarized the extensive investigations in Bohemia, and FURRER[12] has added to those in Switzerland. These all go to prove the validity of the BLYTT-SERNANDER series of climatic periods, and are rapidly reconstructing the details of the vegetation of each period.

Studies in Russia,[13] however, seem to show that the variations in climate were there less clearly defined, although at least one period warmer than the present is evident, with a more northern distribution of such species as *Quercus* and *Pinus*.

Still more recently similar methods, applied by GISTL[14] to some interglacial deposits in Germany, show this last interglacial epoch to have been similar to the postglacial, in having periods corresponding to the preboreal, boreal, atlantic, and sub-boreal with similar vegetation. He estimates the duration of the time represented by these interglacial deposits at 11,000–12,000 years.

These results were similar to those obtained by SZAFER[15] from studies in various localities in central Europe. It would seem that the ice age preceding the interglacial period dealt with was one of a severity much beyond that of the last glacial period.

Finally, WOODHEAD[16] has carefully reviewed the evidence upon which these studies have been based, and concludes that pollen analyses, especially when considered in conjunction with the results from other branches of postglacial research, provide a valuable accumulation of data from which the past history of the forests of Europe may be reconstructed with considerable accuracy. WOODHEAD'S most valuable contribution is a table in which the various researches have been summarized for British Isles, Denmark, Sweden, Norway, Finland, and Switzerland.—G. D. FULLER.

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[11] RUDOLPH, KARL, Die bisherigen Ergebnisse der botanischen Mooruntersuchungen in Böhmen. Beihefte Bot. Centralb. **45**:1–80. 1928.

[12] FURRER, ERNST, Pollenanalytische Studien in der Schweiz. Beiblatt Vierteljahrschr. Naturf. Gesell. Zurich **72**: no. 14. 1–38. 1927.

[13] GERASIMOV, D. A., Climatic changes and forest development during the postglacial period, according to peat bog studies. Bull. Jard. Bot. Princ. U.R.S.S. **25**:319–362. 1926.

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